# Genetic Variation and Attentional and Memory Biases for Emotional Information

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*The mind is not cut in marble*.
George Eliot

This thesis is dedicated to my family, to Hijfte and to friends that matter.

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# List of Abbreviations

| | |
|---|---|
| 5-HT | Serotonin |
| 5-HTT | Serotonin transporter |
| 5-HTTLPR | Serotonin transporter linked polymorphic region |
| AB | Attentional Blink |
| ADRA2B | Alpha 2 adrenergic receptor subtype B gene |
| ANOVA | Analysis of Variance |
| COMT | Catechol-O-methyl transferase |
| Del | Deletion |
| DPSD | Dual Process Signal Detection |
| FAR | False Alarm Rate |
| fMRI | Functional Magnetic Resonance Imaging |
| GSR | Galvanic Skin Response |
| GWAS | Genome wide association studies |
| HWE | Hardy Weinberg Equilibrium |
| HR | Hit Rate |
| IAPS | International Affective Picture System |
| Ins | Insertion |
| ISI | Inter Stimulus Interval |
| MAO | Monoamine oxidase |
| Mcg | micrograms |
| Met | Methionine |
| NART | National Adult Reading Test |
| PFC | Prefrontal cortex |
| PTSD | Post traumatic stress disorder |
| SDT | Signal Detection Theory |
| ROC | Receiver Operating Characteristic |
| SNP | Single nucleotide polymorphism |
| RMANOVA | Repeated Measures Analysis of Variance |
| RPM | Restriction Partition Method |
| RSVP | Rapid Serial Visual Presentation |
| SD | Standard Deviation |
| SNRI | Serotonin and Noradrenaline Reuptake Inhibitor |
| SSRI | Selective Serotonin Reuptake Inhibitor |
| Val | Valine |

# Introduction to Thesis

## *Background*

### Introduction

Antidepressant medications that modulate the levels of brain monoamine neurotransmitters, including serotonin, noradrenaline and dopamine, have been used for over half a century to treat affective disorders. Yet there is limited understanding of how monoamine dysregulation gives rise to the cognitive features of depression or how these medications exert their therapeutic effects at a cognitive level. Furthermore, the fact that up to 70% of patients treated with these medications fail to respond adequately remains a significant challenge in the management of these disorders (Joanna and Irving, 2005; Souery *et al.* 1999). There is evidence that this response may be genetically determined and over the last decade, a variety of studies have begun to explore the pharmacogenomics of antidepressant response (Kato and Serretti, 2010). Yet significant advances in this area have thus far been limited by a failure to produce a consistent pattern of results.

Cognitive models of affective disorders indicate that biased processing of emotional information plays a role in the aetiology and maintenance of symptoms (Leppanen, 2006) and more recent evidence suggests that at least one of the mechanisms by which antidepressant medications exert their therapeutic effects is by modulating emotional processing (Harmer, 2008). However, it is also increasingly recognised that inter-individual variation in responses to emotional stimuli may contribute not only to differences in vulnerability (and resilience) to emotional disorders, but also response to therapeutic agents (Hamann and Canli, 2004). Yet

the neurobiological mechanisms that underlie these individual differences are still not fully understood. It is increasingly accepted that genetic factors explain small but significant amounts of this variability and evidence to date suggests that variation in a number of genes involved in the regulation of central monoamine levels may contribute (Todd *et al.* 2011). Thus, emotional processing may be a mediating factor in the relationship between genetic variation and antidepressant response. Improved understanding of the genetics of emotional processing is therefore likely to contribute to enhanced understanding of individual variation in antidepressant response. Genes involved monoaminergic signalling represent suitable candidates for further investigation of the relationship between the genetics of emotional processing and antidepressant response.

## Emotional processing

For a given organism, emotions comprise a collective change in body and brain states in response to the evaluation of particular events with respect to their significance, i.e., importance for survival. Emotional processes can therefore be considered to include the cognitive processes by which an organism judges and represents the value of internal or external stimuli and responds accordingly. Thus, emotional processing also relates to the cognitive processes of perception, attention and reasoning, forming the basis for learning in animals and humans. Attention refers to the process of selectively concentrating on one aspect of the environment while ignoring other things[1] (Anderson, 2004) whilst perception has been defined as the cognitive process by which organisms interpret and organise sensation to produce a meaningful experience of the world (Lindsay and Norman, 1977).. Perceptual organisation and interpretation of sensory information follows the process of attaining awareness and involves the additional processes of judgement or evaluation

[1] This definition relates to selective attentional processes.

(Adolphs, 2003). Hence, attentional processes may be considered to support perception. The predominant framework utilised in the investigation of emotional processing is a two dimensional model that suggests that stimuli range from negative (unpleasant or aversive) to positive (pleasant or appetitive) along a dimension of valence and from calm (dull) to arousing (exciting) along an arousal dimension (Lang *et al.* 1993). This framework has been utilised in a series of studies that demonstrate preferential (or biased) attention to, and perception of, emotionally salient stimuli in healthy individuals (Anderson, 2005; Anderson and Phelps, 2001; Keil and Ihssen, 2004; Richards and Blanchette, 2004)

Furthermore, emotional experiences are more likely to be remembered than non-emotional ones (Brown and Kulik, 1977). This memory bias for emotional information, termed 'emotional memory', has been demonstrated experimentally in healthy human volunteers using a variety of stimuli including words, pictures, narrated slide shows and film clips (Bradley *et al.* 1992; Heuer and Riesberg, 1990; LaBar and Phelps, 1998; Williams *et al.* 1996). It was initially suggested that this memory bias was entirely consequent to enhanced attentional processing (Easterbrook, 1959). However, this view has since been challenged by behavioural experiments that demonstrated that enhanced memory for emotional events does not occur solely because more attention is devoted to the emotional information during encoding, the initial stage of memory formation (Christianson *et al.* 1991). More recent evidence from pharmacological and functional neuroimaging studies in healthy humans has shown that such memory biases are likely to arise due to the effect of emotional arousal on the process of memory consolidation, the process by which encoded memories become stable over time (McGaugh, 2000). This evidence also suggests that these biases are mediated by the arousal characteristics of the stimuli as opposed to valence *per se*.

## Emotional processing and affective disorders

Early behavioural studies of emotional processing in depression tended to focus on emotion perception (or recognition) and reported a general deficit in the ability to accurately label facial emotional expressions in symptomatic patients compared to healthy controls (Persad and Polivy, 1993; Rubinow and Post, 1992). Other studies suggested that sadness was most often correctly recognised in depression and difficulties in recognition may at least in part be due to a tendency to label any unidentified emotion as sadness (Mandal and Bhattacharya, 1985). Subsequent behavioural studies also examined attentional processes using a wider range of emotional stimuli including words and pictures and have demonstrated a consistent exaggerated attentional bias towards negative compared to neutral stimuli in depressed patients compared to healthy participants (Gotlib *et al.* 2004b; Gotlib *et al.* 2004a). The picture in remitted patients has been less clear with some initial studies failing to find any difference between healthy participants and euthymic patients (Addington and Addington, 1998; Edwards *et al.* 2001; Lembke and Ketter, 2002). However more recent studies have suggested that emotional processing abnormalities persist following remission of acute depressive episodes, thus representing trait markers of the disorder (Bozikas *et al.* 2006; McClure *et al.* 2005; Yurgelun-Todd *et al.* 2000).

Studies of emotional memory in affective disorders, predominantly depression have largely focussed on two main phenomena: state-dependence and mood-congruence (Blaney, 1986). The former refers to the notion that what is remembered during a given mood is at least partly determined by what was learned when previously in that mood. State-dependence thus differs from the usual conceptualisation of emotional memory in that the affective properties of the learned material itself are considered irrelevant. Mood-congruence, on the other hand, implies that some material, by virtue of its affective valence, is more likely to be encoded or retrieved

when congruent with the prevailing mood. This phenomenon also differs from typical conceptualisations of emotional memory, in that it is valence specific, independent of arousal and is dependent on factors other than the stimulus itself. The phenomenon of mood-congruence has been extensively investigated in depressive disorders and several studies have demonstrated that despite overall impaired memory, depressed patients show a bias towards enhanced recognition and recall of negatively valenced information compared to healthy volunteers (Murphy and Sahakian, 2001). By contrast, memory for positive information is reported to be impaired relative to negative and neutral information (Burt *et al.* 1995; Matt *et al.* 1992). Thus, contemporary cognitive models of depression emphasise a systematic negative attentional and recall bias however such models have only recently begun to integrate underlying neurobiological mechanisms including neurochemistry and genetics (Beck, 2008).

## The neuropharmacology of emotional processing

These cognitive-behavioural findings have led to interest in the effect of antidepressant medications on emotional processing. One fruitful approach to developing this understanding has been the investigation of the effect of such medications on emotional processing in healthy volunteers. However, whilst this constitutes a relatively recent approach to understanding the basic mechanisms of therapeutic agents, it has been used for nearly two decades in the domain of cognitive and affective neuroscience to delineate the neurochemistry of human emotional processing. This literature has focussed on the role of the catecholamines (dopamine and noradrenaline) in emotional memory biases. For example, the central role of noradrenaline in emotional memory was confirmed in a seminal study demonstrating the attenuation of emotional memory following the administration of the beta blocker propanolol to healthy volunteers (Cahill *et al.* 1994). Further studies confirmed that

this occurred via central, as opposed to peripheral, mechanisms (van Stegeren *et al.* 1998) and long-term consolidation, rather than encoding, processes (van Stegeren *et al.* 2005). Much of the evidence linking dopamine and emotional processing derives from studies conducted in animals (Greba *et al.* 2001; Greba and Kokkinidis, 2000; Guarraci *et al.* 1999; Guarraci *et al.* 2000; LaLumiere *et al.* 2003; LaLumiere *et al.* 2004). However, a number of recent studies, including work done in our laboratory demonstrates that dopamine is likely to play a similar role in human emotional processing (Takahashi *et al.* 2005; Takahashi *et al.* 2010; Tessitore *et al.* 2002) (Gibbs *et al.* 2007). Crucially, the vast majority of pharmacological studies have demonstrated similar effects of dopaminergic manipulation on memory for both positive and negative emotional information. Whilst the pharmacology of attentional biases for emotional information has been less extensively researched, at least two recent studies conducted in healthy volunteers also suggest a role for both noradrenaline and dopamine in the modulation of such biases (De Martino *et al.* 2008a; Franken *et al.* 2004).

In contrast to the affective neuroscience literature aimed at elucidating the neurobiological mechanisms of emotional processing and focussing on the role of the catecholamines dopamine and noradrenaline in memory, interest in serotonergic mechanisms contributing to human emotional processing has evolved more from experimental medicine approaches aimed at understanding the cognitive mechanisms of antidepressant drug effects. Over the last decade, the ability of both serotonergic and noradrenergic antidepressant drugs to produce biases towards positive information has been documented in studies of emotional processing in healthy individuals (Harmer *et al.* 2008; Harmer *et al.* 2003; Harmer *et al.* 2004; Murphy *et al.* 2009a; Norbury *et al.* 2007). Yet, it is worth noting that one study demonstrated enhanced attention processing of both positive and negative stimuli following administration of a single dose of the noaradrenergic antidepressant reboxetine (De Martino

*et al.* 2008b; Murphy *et al.* 2009b). Furthermore, other studies have found that single doses of the serotonergic antidepressant citalopram enhanced fear processing (Browning *et al.* 2007). It has been suggested these seemingly inconsistent findings may relate to dissociable effects of single versus chronic dosing (Harmer *et al.* 2009). Indeed, evidence from both animal and human studies that acute doses result in an initial enhancement in the processing of negative information that is attenuated with repeated dosing (Burghardt *et al.* 2004; Harmer *et al.* 2003; Harmer *et al.* 2004).

## The genetics of emotional processing

Given the central role of monoamine neurotransmitters (serotonin, dopamine and noradrenaline) in emotional processing as detailed above, efforts to understand neurobiological mechanisms underlying individual differences in emotional processing have focussed on variations in genes involved in monaminergic signalling.

Psychotropic agents targeting the serotonergic system play a central role in the treatment of affective spectrum disorders and are known to modulate emotional processing as described above. Therefore unsurprisingly, one of the genetic variants that have been most extensively investigated in relation to human emotional processing and emotional disorders is the gene encoding the serotonin transporter (5-HTT). An insertion/deletion polymorphism (5-HTTLPR) in the gene promoter region results in two common allelic variants: short (*S*) and long (*L*). The former has been associated with reduced transporter transcription, resulting in approximately 50% reduction in transporter availability in vitro and presumed increased synaptic serotonin availability (Heils *et al.* 1996). The *S* allele has been associated with anxiety-related personality traits that are related to an increased risk for depression (Hariri *et al.* 2005; Pezawas *et al.* 2005; Schinka *et al.* 2004). This polymorphism has also been

associated with differential reactivity of the amygdala, a key brain structure involved in the processing of emotional information. In healthy individuals, the *s* allele has been associated with increased amygdala responsivity to emotional stimuli compared with the *l* allele (Hariri *et al.* 2002; Hariri *et al.* 2005; Heinz *et al.* 2004) (Canli *et al.* 2005). However, whilst these studies have confirmed genotype dependent difference in brain activation during emotional processing, they failed to find any differences in cognitive-behavioural measures. Indeed, it has been suggested that functional neuroimaging studies have greater statistical power to detect genotype dependent effects as they are considered to represent a more proximate biological link to genes (Hariri *et al.* 2006). Therefore, whilst the sample sizes may have been appropriate for detecting such neural differences, it is likely that the detection of behavioural differences would have required much larger numbers. Therefore the behavioural implications of these neural markers remained unclear. Recent studies investigating the potential genetic contribution to attentional biases for emotional stimuli have reported behavioural data suggesting an association between 5-HTTLPR variants attentional biases for emotional stimuli, however this work also remains in the early stages of development (Beevers *et al.* 2007; Beevers *et al.* 2009; Beevers *et al.* 2010; Beevers *et al.* 2011; Osinsky *et al.* 2008).

The contribution of genetically influenced differences in noradrenergic tone to inter-individual variation in human emotional processing and disorders has been little explored. A common variant of the α2b-adrenergic receptor gene (ADRA2B) involves the deletion of three glutamic acid residues in the third intracellular loop region (Small *et al.* 2001). This deletion variant is common across ethnic groups but has been found to be more prevalent in Caucasians (31%) compared to African-Americans (12%). It is associated with two potentially relevant phenotypic changes, relative to the insertion wild-type: (i) a loss of

agonist-promoted desensitization and (ii) a moderate decrease in agonist-mediated receptor function. Thus, its functional consequences, which are still not fully understood, may be related to either of these changes. The functional implications are further complicated by the pre-synaptic and post-synaptic location of the (auto)receptor whereby activation may lead to both potentiation or attenuation of noradrenergic transmission, respectively. However recent evidence suggests that the ADRA2B deletion variant acts primarily as a loss of function polymorphism, resulting in a reduction in the activity of the α2b-adrenergic autoreceptor, leading to potentiation of noradrenergic transmission in an autosomal dominant manner (Rasch *et al.* 2009). A prior study involving a large sample of healthy Swiss volunteers and survivors of the Rwandan genocide, found that the ADRA2B deletion variant was associated with significantly greater emotional enhancement of memory in both groups, as well as increased PTSD-related experiences in the genocide survivors (de Quervain *et al.* 2007). Further studies have demonstrated an association between the ADRA2B deletion variant and increased amygdala activation in response to emotional relative to neutral stimuli (Cousijn *et al.* 2010; Urner *et al.* 2011).

Dopaminergic transmission has played a less central role in the therapeutics of affective spectrum disorders. However, the majority of effective antidepressants display either primary (in the case of the atypical antidepressant bupropion) or secondary dopaminergic effects. Evidence has emerged linking variation in the catecholamine-O-methyltransferase enzyme (COMT) gene, responsible for the degradation of dopamine in the prefrontal cortex to emotional processing. This more recent evidence has emerged as a adjunct to the more extensive body of work linking COMT to cognitive processing (Barnett *et al.* 2008). A single nucleotide polymorphism (SNP) (val158met) that results in the substitution of the amino acid methionine (met) for valine (val) causes a 25 to 75% reduction in the activity of

the COMT enzyme (Chen *et al.* 2004), leading to increased levels of extracellular dopamine in the prefrontal cortex (PFC) and enhanced performance on PFC cognitive tasks including working and episodic memory (de Frias *et al.* 2004; Egan *et al.* 2001). However, evidence has also emerged suggesting that this polymorphism may also play a role in emotional processing. For instance, a number of functional neuroimaging studies have demonstrated exaggerated neural responses to negative but not positive emotional stimuli in COMT met158 carriers (Smolka *et al.* 2005). In fact, in the former study, the number of met alleles was shown to positively correlate to increased amygdala activation for unpleasant negative stimuli. Other behavioural genetics studies examining the role of COMT genetic variation in fear processing using affective startle reflex modulation tasks have also demonstrated that met/met carriers display an exaggerated reaction towards aversive stimuli compare to val/val carriers (Montag *et al.* 2008). Therefore whilst the met allele may be advantageous for cognitive processing, the val allele is advantageous for emotional processing and may possibly confer a resilience to emotional disorders (Heinz and Smolka, 2006). It has been suggested that these pleiotropic effects may be of evolutionary significance, thus contributing to the relatively equal prevalence of both alleles within populations (Mier *et al.* 2009).

The enzyme monoamine oxidase A (MAOA) is involved in the degradation of all three monoamines (noradrenaline, dopamine and serotonin and therefore also plays a critical role in the regulation of their neurotransmission. A variable number of tandem repeats (VNTR) polymorphism gene coding for MAOA selectively influences the transcriptional activity of the MAOA gene promoter such that enzyme expression is relatively high for carriers of the 3.5-repeat or 4-repeat alleles (MAOA *H*) and lower for carriers of 2, 3, or 5 repeats (MAOA *L*). Thus MAOA *L* individuals are predicted to have higher brain monoamine levels and this has been linked to increased amygdala activation in healthy individuals during processing of

unpleasant stimuli, compared to the high expression (MAOA *H*) genotype (Lee and Ham, 2008; Meyer-Lindenberg *et al.* 2006). A further study has also confirmed links between this polymorphism and emotional neural correlates of processing in the normal population (Williams *et al.* 2009). However, none has reported any cognitive/behavioural measures of emotional processing.

## *Scope*

### Rationale

Despite significant advances in the integration of cognitive and neurobiological models of emotional processing, as described above, understanding of the genetic determinants of inter-individual variation remains rudimentary. For example, behavioural genetics studies have tended to focus on the effects of single genes within a given study/sample and although they are generally considered to act synergistically, few studies have specifically investigated interactions between these polymorphisms. However, it has been suggested that gene-gene interactions (or epistasis) are likely to be of greater importance in predicting effects, particularly in complex traits and disorders (Elvevåg and Weinberger, 2009). Consequently, important effects might be missed if the genes are examined in isolation without allowing for potential interactions with other known or unknown genes or environmental factors. This may account for some of the inconsistencies in single-gene association studies including non-replication of findings.

Thus, whilst the evidence to date suggests that the genes involved in monaminergic neurotransmission (5-HTTLPR, ADRA2B, MAOA and COMT) may contribute to an increased risk of affective disorders via their effects on emotional processing, little is known

about how the different variants of these genes might interact within a given individual to influence these outcomes. Given the interactive relationships between these neurotransmitter systems in the brain, it is very likely that these genes also interact to produce their effects. In fact, such effects have begun to be explored. For example, one of the only recent studies to examine multiple gene effects, established that the COMT *met*158 and 5-HTTLPR *S* alleles, as well as an additional 5-HTTLPR SNP variant (*G*) allele, produced an additive effect in enhancing neural responses to emotional stimuli (Smolka *et al.* 2007).

The purpose of this thesis was to extend understanding of the impact of genetic variation on emotional processing in healthy individuals. Studying healthy volunteers provides the opportunity to understand the neurobiology of emotional processing, likely to be relevant to therapeutic advances, in the absence of likely confounders in clinical populations such as concomitant or previous treatment, the disease process itself, and other factors that may be subject to increased variability in a patient population, such as intelligence and motivation (Gilles and Luthringer, 2007). Given that attention and memory biases for emotional information are a well established feature of emotional processing with known neural correlates, these were used as the marker of cognitive performance. The candidate genes of interest (COMT, 5-HTTLPR, ADRA2B and MAOA) were selected on the basis of evidence of links to behavioural and/or neural correlates of emotional processing based on review of the existing literature detailed above. Additionally, given that the brain derived neurotrophic factor gene (BDNF) *val*66*met* polymorphism has also been suggested as a predictor of episodic memory, this genotype will also be determined to ensure that the results are not confounded by variation in this gene (Egan *et al.* 2001).

## Aims

The general aim study was to investigate the effects of the candidate genetic polymorphisms (COMT *met*158, 5-HTTLPR *S*, ADRA2B *Del* and MAOA *L*) on cognitive measures of emotional processing.

The specific aims were:

(i) to examine the independent contribution of each polymorphism (single-gene effects)

(ii) to conduct a preliminary exploration of epistasis (gene-gene interactions)

## Hypotheses

Single-gene effects

The primary hypothesis was that COMT, 5-HTTLPR, ADRA2B and MAOA genotype would each exhibit a main effect on attentional and memory biases for emotional information. It was predicted that the variants that have been associated with increased amygdala reactivity (specifically COMT *met*158, 5-HTTLPR *S*, ADRA2B *Del* and MAOA *L*) would also be associated with increased attentional and memory biases for emotional, relative to neutral information. We predicted that these biases would increase with increasing numbers of 'emotional bias alleles' (COMT *met*158, 5-HTTLPR *S*, ADRA2B *Del* and MAOA *L*).

Gene-gene interactions (epistasis)

As each of these alleles should independently affect monoamine levels, it was hypothesised that epistatic effects would be synergistic, i.e. attentional and memory biases for emotional information will be correlated with total number of 'emotional bias alleles' (COMT *met*158, 5-HTTLPR *S*, ADRA2B *Del* and MAOA *L*).

## Sample size

The frequencies of the COMT *met*158 and 5-HTTLPR *S* are both in the region of 50% in the population to be studied (Egan *et al.* 2001). Hence the following approximate genotype frequencies were expected (*val*/*val*, 25%; *val*/*met*, 50%; *met*/*met*, 25% and *L*/*L*, 25%; *L*/*S*, 50%; *S*/*S*, 25%), respectively. The prevalence of the deletion variant of ADRA2B is 33% (Small *et al.* 2001) hence we expected 55% of the sample to be deletion carriers (including homozygous and heterozygous) and 45% to be non-carriers. For MAOA, the allele frequencies are *H* (65%) and *L* (35%) and similar genotype frequencies would be expected in the hemizygous male sample.

Based on these allele frequencies, power calculations were conducted based on a 5% level of significance. A total sample size of 100 would provide 99% power to detect statistically significant main effects of COMT and 5-HTTLPR genotypes, and 75% and 65% power to detect statistically significant effects of ADRA2B and MAOA, respectively. There are a number of limitations to this power analysis. Firstly, given that the effect sizes were not known, an estimated moderate effect size of 0.5 was used. Furthermore, it was not been possible to model gene-gene interactions in the power calculations due to the complexity of such interactions and the lack of exiting data. Nevertheless, the power analysis indicates that the present study design is likely to provide sufficient power to detect the effects of individual genes. Examination of gene-gene interactions will therefore be exploratory in the first instance, but will form the basis for future study design.

## *Overview of thesis*

Our study led to 3 published articles which form the basis of this thesis. In the first two, we report on the genetic variation and memory bias component of the study (Gibbs *et al.* 2010; Naudts *et al.* 2012a). In the third one, we report on the genetic variation and attention bias arm of the study (Naudts *et al.* 2012b). Thus, the 3 articles concern different aspects of the same study on the same 107 participants, who all participated in the same procedure as explained below. Due to subject drop-out and genotyping failure, the sample size in the first 2 articles was reduced to 97 and to 94 in the 3rd article**.**

## Participants

Healthy male volunteers of White British ethnicity between the ages of 18 and 35[2] via the Mindsearch database were recruited to the study. Mindsearch is a service that connects academic researchers at the King's College London with healthy volunteers from the general public to participate in their research projects. The database is run by an administrator who maintains the database, identifies potential volunteers for researchers and undertakes out-reach activities to involve the local community in research. It is funded by the National Institute for Health Research Biomedical Research Centre (NIHR-BRC), as well as by making a small charge for researcher using the database, and has over 4,000 volunteer members. There is no cost to volunteers to be included in the database and they usually receive payment for participation in research.

The narrow age range was chosen to reduce the likelihood of valid associations being obscured by age differences and the restriction on ethnicity was to reduce population stratification effects. Recruitment was limited to male participants. This gender selection also has the advantage of avoiding confounding by the now well-established gender differences in

[2] The 40 stated in Article 2 is a typographical error

emotional processing (Cahill, 2006). A database of 318 volunteers meeting these requirements was provided by Mindsearch. Eligible participants were contacted for possible recruitment to the study. They were further screened to exclude any history of psychiatric or neurological disorder using a checklist questionnaire (Appendix I). 107 participants meeting the inclusion criteria were recruited to the study, including 7 additional participants beyond the planned sample of 100, to compensate for drop-outs.

## Procedure

On arrival, following informed consent, buccal swabs were collected from volunteers for external genotyping by KBioscience. Of the 107 samples collected, genetic data were successfully obtained for 106 for ADRA2B, 105 for COMT, 96 for 5-HTTLPR and 106 for BDNF. Due to technical issues with the PCR for the MAOA assay, this polymorphism was excluded from analysis. The National Adult Reading Test (Nelson, 1982) was administered in order to obtain an estimate of IQ and the encoding phase of the Emotional Memory (EM) Task was administered, followed by the Attentional Blink (AB) Task. Participants returned 1 week later for the recognition memory phase of the EM task. Seven participants failed to return for the recognition memory test. Further details on procedure and methods can be found in the articles.

# Article 1. Deletion variant of α2b-adrenergic receptor moderates the effect of COMT val$^{158}$met polymorphism on episodic memory performance

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**Key words:**

Genetics; emotional memory; episodic memory; COMT; ADRA2B, catecholamines; noradrenaline

## *Abstract*

The COMT *val*$^{158}$ variant has been associated with impaired cognitive function compared to the *met*$^{158}$ variant yet gene-gene interactions are not well described. In this study we demonstrate an interaction between this COMT polymorphism and a deletion variant of ADRA2B, the gene encoding the α2b-adrenergic receptor on episodic memory performance. Specifically, carriage of the ADRA2B deletion abolished the relative memory impairment in homozygous COMT *val*$^{158}$ carriers compared to *met*$^{158}$ carriers.

## *Introduction*

Animal studies suggest that memory formation is dependent on noradrenergic modulation of the process of long-term potentiation (LTP) in the hippocampus (Straube and Frey, 2003). Other work also implicates the dopamine system of the prefrontal cortex in modulating memory storage (Williams and Goldman-Rakic, 1995). Enhancement of memory for emotionally arousing events in animals and humans has been linked to an increase in central noradrenergic transmission, mediated via the amygdala and its projections to the hippocampus and other brain regions, such as the dopamine system of the prefrontal cortex (Roozendaal *et al.* 2009). Amygdala lesions in animals have been demonstrated to result in an attenuated stress-induced increase in dopamine turnover in the prefrontal cortex (Davis *et al.* 1994). This suggests that the effect of emotional arousal on memory may depend in part, on noradrenergic amygdala-influenced effects on the dopamine-related activity of the prefrontal cortex. This is supported by functional neuroimaging data highlighting the importance of interaction between these brain regions during emotional processing (Keightley *et al.* 2003). More recently, the function of both of these regions has been related to genetic variation (Smolka *et al.* 2005).

A deletion variant of the ADRA2B gene results in reduced functionality of the α2b-adrenergic autoreceptor and presumed potentiation of central noradrenergic transmission. This variant has been associated with enhanced emotional memory in humans (de Quervain *et al.* 2007). A G to A missense variant of the COMT gene (*met* $^{158}$) results in reduced functionality the catechol-O-methyl transferase enzyme responsible for the degradation of dopamine in the prefrontal cortex and presumed higher extracellular dopamine levels (Chen *et al.* 2004). This variant has been associated with enhanced episodic memory as well as

exaggerated amygdala and prefrontal cortical responses to emotional stimuli relative to the *val*$^{158}$ allele (de Frias *et al.* 2004; Smolka *et al.* 2005). However, no studies have so far investigated the effects of interaction between these gene systems on memory formation.

In this study we set out to determine whether the ADRA2B and COMT genotypes would interact to modulate emotional and non emotional memories. We predicted that episodic memory performance would be better in COMT *met*$^{158}$ carriers and this variant would produce additive effects in combination with the ADRA2B deletion variant to produce greater emotional enhancement of memory.

## *Experimental Procedures*

Memory testing and genotyping in relation to the ADRA2B deletion and COMT val$^{158}$met polymorphisms were carried out in 97 Caucasian healthy male volunteers aged 18 – 35 years (mean = 24.1, SD = 4.8). The study was approved by the King's College London Research Ethics Committee and all participants gave written informed consent. During the encoding phase participants viewed 92 pictures from the International Affective Picture System (IAPS)(Lang *et al.* 1998). Half of them were negative-arousing (mean valence = 2.6, SD = 0.9 and mean arousal = 6.1, SD = 0.6) and half were neutral (mean valence= 5. 1, SD = 0.6 and mean arousal = 3.3, SD = 0.8). The pictures were presented on a laptop computer for 3 seconds with a 4 second inter-stimulus interval (ISI), during which a fixation cross was present on the screen. The order of presentation was randomized across participants. Estimates of verbal IQ were derived from the National Adult Reading Test (NART). Delayed memory was tested 1 week later when participants returned for an unexpected recognition memory test in which they viewed all of the 92 previously seen pictures and 92 foils matched for content, valence and arousal. After each picture, participants made an "old"/"new"

judgment and rated the arousal and valence of each picture using a 9-point scale. The hit rate (HR) and false alarm rate (FAR) were calculated for each participant and memory accuracy was assessed by the calculation of the discrimination index ($P_r$) based on two-high-threshold theory (Snodgrass and Corwin, 1988).

DNA extraction from buccal swabs was carried out by KBiosciences using their internal GuSCN-based extraction protocol and genotyping was carried out using their PCR SNP genotyping system （KASPar®). 1.5ul DNA (@ ~10ng/ul) per well, dried down before PCR onto KBioscience 384-well plates, 4ul PCR volume (using 2x KASPar genotyping system reagent) at 94degC for 15min (94degC for 10sec, 57degC for 60s) x36 cycles. Plates were read using a BMG PheraStar microtitre plate fluorescence reader.
Two forward primers and one reverse primer were used as follows for ADRA2B:
pF1:GAAGGTGACCAAGTTCATGCTCCTCCTCCTCCTCCTCTTCA (detects 'Short' allele) and
pF2:GAAGGTCGGAGTCAACGGATTCCTCCTCCTCCTCCTCTTCC (detects 'Long' allele)
pR:GAAGGAGGGTGTTTGTGGGGCAT and COMT:
pF1:GAAGGTGACCAAGTTCATGCTGGCATGCACACCTTGTCCTTCAT (detects 'A' allele)
pF2:GAAGGTCGGAGTCAACGGATTGCATGCACACCTTGTCCTTCAC (detects 'G' allele)
pR:CATCACCCAGCGGATGGTGGAT

## *Results*

Of the 97 participants, 9 were homozygous carriers of the ADRA2B deletion, 48 heterozygotes and 41 did not carry the deletion. Due to the small number of homozygous carriers, they were combined with the heterozygotes. 35 participants were homozygous for the *met*$^{158}$ allele of the COMT gene, 25 were homozygous for the *val*$^{158}$ allele and 37 were heterozygous. These frequencies did not deviate from Hardy-Weinberg equilibrium. Repeated measures analysis of variance revealed that memory accuracy as measured by $P_r$ was significantly greater for negative-arousing pictures than neutral pictures [F = 29.0, degrees of freedom = 1, error degrees of freedom = 91, $p < 0.001$]. However there was no effect of ADRA2B or COMT genotype on emotional memory as assessed by the absence of both 2-way and 3-way arousal x genotype interactions. However, there was a highly significant interaction between ADRA2B and COMT genotype [$F(2, 91) = 6.7$, $p = 0.002$] accompanied by a trend towards a significant main effect of COMT on overall memory performance [$F(2, 91) = 2.7$, $p = 0.07$] (Fig 1). Mean memory performance measures (hit rate, false alarm rate and $P_r$) for aversive and neutral stimuli by genotype are given in Table 1. There were no group differences in age or IQ.

## *Discussion*

In line with previous studies, our results are indicative of episodic memory impairment in carriers of the COMT *val* $^{158}$ allele relative to the *met*$^{158}$ allele. However, we demonstrate for the first time that this disadvantage is abolished by possession of the ADRA2B deletion variant. This is the first demonstration of an effect of ADRA2B genotype on episodic memory performance and its interaction with COMT. This suggests that genes that do not exert direct effects on cognition might do so indirectly via gene-gene interactions. Our hypotheses relating to the effects on emotional enhancement of memory were not confirmed.

The enhanced cognitive performance associated with COMT *met*$^{158}$ allele carrier status has been attributed to enhanced dopaminergic, rather than noradrenergic transmission in the prefrontal cortex (Tunbridge *et al.* 2006). However, prior studies described above have indicated that noradrenergic transmission in other brain regions may play a role in modulating this effect. Our findings suggest that enhanced noradrenergic transmission as a result of reduced function of the α2b-adrenergic autoreceptor may compensate for the reduction in dopamine related activity of the prefrontal cortex associated with the higher activity COMT *met*$^{158}$ allele. However, the neuroanatomical substrate for this effect remains unclear.

We also demonstrated a reduction in memory performance in ADRA2B deletion carriers relative to non deletion carriers in COMT *met*158 allele heterozygotes. This may be related to the observed inverted-U-shaped relationship between catecholamine levels and pre-frontal cortical function (Meyer-Lindenberg *et al.* 2005). Given that *met*/*met* individuals are hypothesised to have near-optimal central catecholamine function, increased noradrenergic transmission via the ADRA2B deletion may result in a relative impairment in memory performance.

One limitation of this study is the relatively small sample size that may have contributed to our failure to demonstrate arousal x genotype effects. This failure may also be related to the use of recognition memory testing as opposed to recall. Although emotional arousal may lead to enhanced recall of information, it may not always lead to increased accuracy on recognition memory testing (Windmann and Kutas, 2001). This has been attributed to an emotion-induced sense of familiarity, leading to a more liberal response bias, i.e. an increased

tendency to classify stimuli as previously seen, irrespective of whether they have been or not (Dougal and Rotello, 2007). Under such circumstances, the response bias criterion may provide a better marker of emotional modulation of memory.

Recent pharmacogenetic approaches to therapeutic drug development for neuropsychiatric disorders have begun to examine the interaction between genetic variation and cognitive response. For example, pre-clinical studies in healthy volunteers indicate that the COMT inhibitor tolcapone interacts with COMT genotype in producing effects on cognition, such that *val/val* genotypes exhibit improvement and *met/met* genotypes worsen (Apud *et al.* 2006). This is consistent with our findings which may have implications for future neuropharmacological attempts to enhance cognition via the noradrenergic system.

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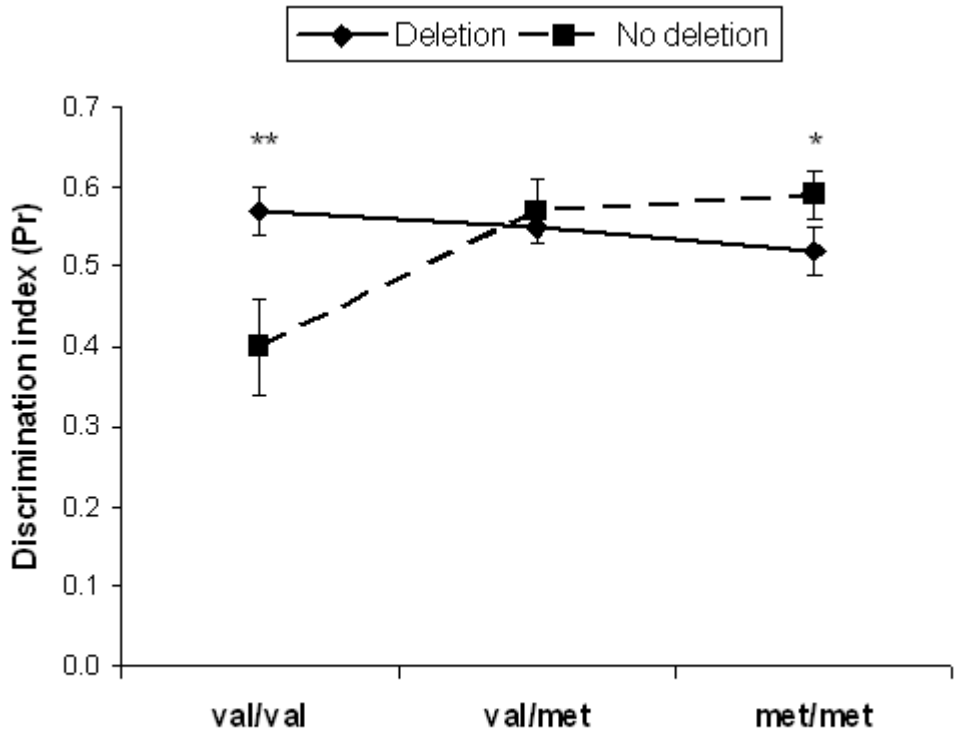
Table 1: Mean memory performance measures (hit rate, false alarm rate and $P_r$) for aversive and neutral stimuli by genotype.

| *Performance Measure* | *ADRA2B* | *COMT* | *Stimulus* | *Mean* | *SD* |
|---|---|---|---|---|---|
| Hit Rate | No deletion | *val/val* | Aversive | .70 | .23 |
| | | | Neutral | .55 | .21 |
| | | *val/met* | Aversive | .80 | .12 |
| | | | Neutral | .69 | .12 |
| | | *met/met* | Aversive | .82 | .08 |
| | | | Neutral | .69 | .12 |
| | Deletion | *val/val* | Aversive | .81 | .10 |
| | | | Neutral | .69 | .14 |
| | | *val/met* | Aversive | .80 | .11 |
| | | | Neutral | .63 | .14 |
| | | *met/met* | Aversive | .76 | .13 |
| | | | Neutral | .62 | .14 |
| False Alarm Rate | No deletion | *val/val* | Aversive | .25 | .13 |
| | | | Neutral | .21 | .13 |
| | | *val/met* | Aversive | .22 | .15 |
| | | | Neutral | .15 | .14 |
| | | *met/met* | Aversive | .18 | .07 |
| | | | Neutral | .13 | .07 |
| | Deletion | *val/val* | Aversive | .21 | .13 |
| | | | Neutral | .14 | .08 |
| | | *val/met* | Aversive | .19 | .10 |
| | | | Neutral | .11 | .07 |
| | | *met/met* | Aversive | .19 | .08 |
| | | | Neutral | .15 | .10 |
| Discrimination Index (Pr) | No deletion | *val/val* | Aversive | .46 | .16 |
| | | | Neutral | .35 | .20 |
| | | *val/met* | Aversive | .58 | .19 |
| | | | Neutral | .53 | .16 |
| | | *met/met* | Aversive | .64 | .11 |
| | | | Neutral | .56 | .15 |
| | Deletion | *val/val* | Aversive | .60 | .16 |
| | | | Neutral | .55 | .12 |
| | | *val/met* | Aversive | .61 | .11 |
| | | | Neutral | .52 | .11 |
| | | *met/met* | Aversive | .57 | .14 |
| | | | Neutral | .46 | .16 |

**Figure legends**

Figure 1

Discrimination index ($P_r$) for all items. There was no difference in memory performance between COMT genotypes in those with the ADRA2B deletion. However, in the absence of the deletion memory performance of homozygous COMT *val*$^{158}$ carriers was significantly worse than carriers of the *metl*$^{158}$ allele. ** $p < 0.01$,* $p < 0.05$.



# Article 2. Influence of COMT val158met and ADRA2B deletion polymorphisms on recollection and familiarity components of human emotional memory

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## *Abstract*

Emotional enhancement of memory is a widely accepted phenomenon that, in addition to its adaptive role, may play a role in the evolution of psychiatric disorders. Hence a comprehensive understanding of its neurobiological basis is imperative. Whilst the pharmacological and neural mechanisms are well known, the contribution of genetic variation is not. Research suggests that two qualitatively different processes (recollection and familiarity) contribute to recognition memory. In this study, we examined the relative contribution of two common genetic polymorphisms, the deletion variant of the ADRA2B gene that codes the α2b adrenergic receptor and the val158met polymorphism of the COMT gene that codes the catechol-O-methyltransferase enzyme, to emotional enhancement of these two memory processes in 97 healthy male volunteers. There was a significant interaction between COMT genotype and emotional arousal in relation to recollection, but not familiarity, with the former being significantly elevated for emotionally arousing *vs*. neutral pictures in carriers of the val158 allele compared to met158 carriers. There were no main effects or interactions in relation to ADRA2B genotype.

**Key words:**

Genetics; emotional memory; episodic memory; COMT; ADRA2B, catecholamines; noradrenaline

## *1. Introduction*

It is well established that memory is enhanced for emotional compared to neutral events and experiences (Brown and Kulik, 1977). Whilst this phenomenon, termed 'emotional memory', has long been held to confer an evolutionary advantage related to the enhanced retention of information relevant to survival, there is increasing evidence that it may also contribute to psychopathological processes. For example, the intrusive reliving of traumatic memories associated with post-traumatic stress disorder (PTSD) is considered to be a maladaptive consequence of emotional memory processes (Elzinga and Bremner, 2002;Labar and Cabeza, 2006). Such processes are also hypothesised to contribute to abnormal belief formation in psychotic disorders (Fotopoulou, 2010;Gibbs and David, 2003). Thus, understanding the mechanisms of emotional memory formation and maintenance has become an increasingly important domain of cognitive neuroscience research.

Emotional memory has been investigated experimentally using a variety of stimuli including words (Kleinsmith and Kaplan, 1963), pictures (Bradley et al., 1992) and stories (Cahill and McGaugh, 1995), although the extent to which these laboratory paradigms accurately reflect real-life emotional memories has been questioned (Todd et al., 2011). Nevertheless, a wealth of research using these paradigms has elucidated many of the neurobiological mechanisms underlying emotional enhancement of memory. For example, initial lesion studies in humans demonstrated that the amygdala is a key neural substrate for emotional enhancement of memory (Adolphs et al., 1997;Cahill et al., 1995) and this has been confirmed in multiple subsequent functional neuroimaging studies (Cahill et al., 1996;Canli et al., 1999;Canli et al., 2000;Hamann et al., 1999). Pharmacological challenge studies with beta blockers and dopamine antagonists in healthy human volunteers have demonstrated the crucial role of the

catecholamine neurotransmitters noradrenaline and dopamine (Cahill et al., 1994;van Stegeren et al., 1998;van Stegeren et al., 2002;Gibbs et al., 2007) and multiple functional magnetic resonance imaging (fMRI) studies have confirmed the role of noradrenergic transmission in amygdala mediated consolidation of emotional memories (van Stegeren et al., 2005;van Stegeren, 2008). Although similar pharmacological fMRI studies have not yet been conducted in relation to the effects of dopamine on emotional memory, there is evidence that dopamine is involved in modulating the human amygdala response (Takahashi et al., 2010;Takahashi et al., 2005;Tessitore et al., 2002) and its contribution to emotional memory has been established in a plethora of animal studies (Greba et al., 2001;Guarraci et al., 1999;Guarraci et al., 2000;LaLumiere et al., 2004).

However, the contribution of genetic variation to human emotional memory has only recently begun to be investigated. As yet, little is known about the factors underlying individual differences in emotional memory that might contribute to individual vulnerability or resilience to psychological disorders such as PTSD (Todd and Anderson, 2009). Consistent with existing literature, it has been hypothesised that genes contributing to noradrenergic and dopaminergic neurotransmission (ADRA2B and COMT) are likely to contribute to individual differences in emotional enhancement of memory (Todd et al., 2011). For instance, a polymorphic deletion in the ADRA2B gene has been linked to emotional memory. This deletion results in reduced agonist–promoted desensitisation of the α2b adrenergic autoreceptor *in vitro* and is therefore presumed to contribute to potentiation of noradrenergic transmission (Small et al., 2001). Although this has not been directly demonstrated *in vivo*, indirect behavioural and neuroimaging data support this assumption. Two studies have demonstrated that recall of emotionally arousing, compared to neutral images is enhanced in ADRA2B deletion carriers relative to non carriers (de Quervain et al., 2007) and this is

associated with increased amygdala activation during encoding (Rasch et al., 2009). The finding that the deletion variant was also associated with increased PTSD symptoms highlights the potential clinical relevance of genetically influenced emotional memory. However, two factors limit the conclusions that can be drawn about the role of ADRA2B in long-term emotional enhancement of memory from these two seminal studies. First, memory was tested after a very short (10 minute) retention interval and second, the observed genotype-related differences in amygdala activation during encoding were not associated with subsequent memory for the emotional stimuli. Thus, the contribution of ADRA2B to longer-term consolidation processes remains unclear. On the other hand, COMT has not been directly linked to emotional memory although the relationship between the COMT polymorphism and dopamine levels has been demonstrated *in vivo* (Gogos et al., 1998). A common polymorphism (val158met) in this gene, the substitution of methionine (met) for valine (val) results in a functional reduction in the activity of the enzyme, catechol-O-methyltransferase (COMT), responsible for the degradation of dopamine in the prefrontal cortex. The met allele is associated with 40% less COMT activity (Chen et al., 2004), resulting in higher levels of dopamine in the prefrontal cortex. However the behavioural effects of this polymorphism are complicated by increasing evidence of pleiotropy[3] (Mier et al., 2009). For example, initial studies suggested that the met allele is associated with enhanced prefrontal cognitive function, particularly executive function (Egan et al., 2001;Bertolino et al., 2006), but also episodic memory (de Frias et al., 2004). However, other evidence suggests that the COMT val158met polymorphism may also play a role in individual responses to emotional stimuli. A number of more recent studies suggest that the met allele is also associated with an increased neural response to aversive, relative to neutral and positive stimuli, specifically involving the amygdala and prefrontal cortex (Herrmann et

[3] Pleiotropy refers to the process by which a single gene exerts multiple behavioural effects.

al., 2009;Drabant et al., 2006;Smolka et al., 2007;Smolka et al., 2005). Thus, although there is no behavioural or neuroimaging evidence directly linking the COMT val158met polymorphism to emotional memory, its role in the reactivity of brain regions crucial to the emotional enhancement of memory is indicative.

It has therefore been suggested that both ADRA2B and COMT are likely to play a role in individual variation in emotional memory, with ADRA2B contributing to emotional enhancement of consolidation processes by influencing the amygdala and hippocampus, whilst COMT's role may be more likely to relate to prefrontal regulation of amygdala responses to emotional (aversive) stimuli (Todd et al., 2011). This is consistent with the 'modulation hypothesis' suggesting that emotional enhancement of memory occurs via interaction between in prefrontal-amygdala-hippocampal networks. This is supported by existing fMRI data demonstrating that subsequent memory effects for emotionally arousing stimuli can be predicted by correlated activation between these regions during encoding (Dolcos et al., 2004;Kilpatrick and Cahill, 2003;Richardson et al., 2004). Few studies have as yet attempted to investigate potential interactive (epistatic) effects of these genes on emotional memory. We recently began to address this gap by investigating epistasis between the ADRA2B deletion and COMT val158met polymorphisms on emotional memory in healthy human volunteers. We found a significant interaction between the two polymorphisms in relation to overall episodic memory performance. Specifically, ADRA2B deletion carriers who were homozygous for the COMT val allele showed better memory for both neutral and aversive stimuli than non carriers, suggesting that possession of the deletion variant may moderate the cognitive impairments associated with the COMT val allele (Gibbs et al., 2010b). We hypothesised that this is related to the putative inverted "U" that has been

proposed to model the effects of catecholamine levels on prefrontal cognitive function (Meyer-Lindenberg et al., 2005)(see Figure 1).

[Figure 1 about here please]

Ostensibly, by increasing prefrontal noradrenaline levels, the ADRA2B deletion variant shifts high activity COMT val/val individuals to more optimal catecholamine levels and consequently, improved cognitive performance. However, unlike de Quervain et al, we did not find an effect of either gene on emotional enhancement of memory *per se* (de Quervain et al., 2007). This may have been due to reduced power in our smaller sample which would be consistent the absence of behavioural differences in their fMRI study, presumed to be due to the smaller sample size (Rasch et al., 2009). However, the discrepancy may have been due to methodological differences. For example, in order to explore the contribution of genetic variation to longer-term consolidation processes, we examined memory after a delay of one week, rather than the ten minute interval used by de Quervain et al. We also used an "old-new" recognition memory test, as opposed to free recall, in order to examine other processes that may be influenced by emotional arousal, such as memory confidence. In such *one-interval* designs, measures of accuracy or *discrimination* (d') may be derived from *single-process* memory models based on signal detection theory (SDT) (Green and Swets, 1966). In these models d' is considered to relate to a single process of memory strength, or familiarity, from which confidence judgements arise directly (Egan, 1958). However, *dual-process signal detection (DPSD)* models have increasingly been used to account for data indicating that two distinct processes may contribute to recognition memory: recollection and familiarity (Yonelinas, 2002). The latter is considered to be a quantitative, memory-strength based process, associated with a "sense of having previously seen". By contrast, recollection is

presumed to be a qualitative threshold process, associated with high levels of contextual detail and high confidence judgements, and is considered most closely related to recall performance (Mandler, 1980). It is possible that differential effects of genotypes on emotional enhancement of these two different memory processes may account for the difference between our findings and those of de Quervain et al 2007. Therefore, in the present study, we used a DPSD approach to investigate the relative contribution of variation in the ADRA2B and COMT genotypes (including possible interactions) to the recollection and familiarity components of emotional memory.

## *2. Materials and methods*

### 2.1. Participants

One hundred and seven healthy white British male volunteers, aged 18 to 40 years (mean age = 24.1, SD = 4.8) were recruited through an advertisement and received financial compensation for their participation. Potential participants were screened for psychiatric or neurological disorder by means of a checklist questionnaire. Exclusion criteria were (i) any current or past history of psychiatric illness, (ii) any significant history of substance misuse, including nicotine, and (iii) taking regular medication. Estimates of verbal IQ were derived from the National Adult Reading Test (NART) (Nelson, 1982). The study was approved by the King's College London Research Ethics Committee and all participants gave written informed consent.

### 2.2. Genetic Testing

This was carried out as previously reported (Gibbs et al., 2010b). Specifically, DNA extraction from buccal swabs was carried out by KBioscience using their internal GuSCN-

based extraction protocol and genotyping was carried out using their PCR SNP genotyping system （KASPar®). 1.5ul DNA (@ ~10ng/ul) per well, dried down before PCR onto KBioscience 384-well plates, 4ul PCR volume (using 2x KASPar genotyping system reagent) at 94degC for 15min (94degC for 10sec, 57degC for 60s) x36 cycles. Plates were read using a BMG PheraStar microtitre plate fluorescence reader. Two forward primers and one reverse primer were used as follows for ADRA2B:

pF1:GAAGGTGACCAAGTTCATGCTCCTCCTCCTCCTCCTCTTCA (detects'Short' allele) and

pF2:GAAGGTCGGAGTCAACGGATTCCTCCTCCTCCTCCTCTTCC (detects 'Long' allele)

pR:GAAGGAGGGTGTTTGTGGGGCAT and COMT:

pF1:GAAGGTGACCAAGTTCATGCTGGCATGCACACCTTGTCCTTCAT (detects 'A' allele)

pF2:GAAGGTCGGAGTCAACGGATTGCATGCACACCTTGTCCTTCAC (detects 'G' allele)

pR:CATCACCCAGCGGATGGTGGAT

2.3. Emotional Memory Task

We used an emotional memory task similar to that previously used by ourselves and others (de Quervain et al., 2007;Gibbs et al., 2007;Gibbs et al., 2010b;Rasch et al., 2009). During the encoding phase participants viewed 92 pictures from the International Affective Picture System (IAPS) stimulus set (Lang et al., 1998). Half were aversive-arousing (mean valence = 2.6, SD = 0.9 and mean arousal = 6.1, SD = 0.6) and half were neutral (mean valence= 5. 1, SD = 0.6 and mean arousal = 3.3, SD = 0.8). Evidence suggests that the effects of emotional arousal on memory are larger and more consistently observed for aversive compared to

positive emotional stimuli (Kensinger and Corkin, 2004;de Quervain et al., 2007;Rasch et al., 2009). Additionally, the evidence to date suggests that COMT is associated with altered processing of aversive, but not positive stimuli. Hence we chose to use only aversive emotional stimuli given that we were interested in the effects of arousal, rather than valence *per se*. The pictures were presented on a laptop computer for 3 seconds with a 4 second inter-stimulus interval (ISI), during which a fixation cross was present on the screen. The order of presentation was randomised across participants. Participants were instructed to observe the picture while it was being presented and make a binary judgement as to whether they considered the image emotionally arousing or non arousing by pressing one of two laptop keys. Delayed memory was tested one week later when participants returned for an unexpected recognition memory test in which they viewed all of the 92 previously seen pictures and 92 foils matched for content, valence and arousal. Participants were required to judge whether each picture was “old” (previously seen) or “new” (not previously seen) and then rate their confidence in this judgement using a scale ranging from “uncertain” to “very certain”. In order to reduce the likelihood of participants making only high-confidence or low-confidence judgements, they were advised to make use of the entire scale. Participants were also asked to rate each picture for arousal and valence on scales ranging from “calm” to “aroused” and “unpleasant” to “pleasant”, respectively, in line with previous approaches (Lang et al., 1988)

2.4. Statistical Analysis

Hit rates (proportion of previously seen items correctly identified as ‘old’) and false alarm rates (proportion of foils incorrectly identified as ‘old’) for each stimulus category were calculated for each participant. Estimates of recollection (R) and familiarity (d') for each participant were derived from receiver operating characteristic (ROC) curves generated by

plotting performance (hit rate *vs*. false alarm rate) as a function of response confidence. This was carried out using a Microsoft Excel solver that used model dual-process equations to reduce the sum of squared errors between the predicted and observed data (Yonelinas et al., 1998). The number of items in each confidence category was examined to ensure that participants had followed the instructions to utilise the entire confidence scale. This was necessary as evidence suggests that failure to use the entire scale leads to ROC points that are closely clustered together, making it difficult to accurately assess the function.

## *3. Results*

### 3.1. Genotypes

Of the 107 participants, ADRA2B genotypes were missing for 2 participants, 11 were homozygous carriers of the ADRA2B deletion, 48 were heterozygotes and 46 were non carriers. These frequencies did not deviate from Hardy-Weinberg equilibrium ($X^2$=0.09, p=0.77). Due to the small number of homozygous carriers, they were combined with the heterozygotes, giving two genotype groups of deletion carriers (del) and non-carriers (no del) as previously done by de Quervain et al 2007. COMT genotype was missing for 1 participant. Thirty-six participants were homozygous for the met158 allele, 27 were homozygous for the val158 allele and 43 were heterozygous, consistent with Hardy-Weinberg equilibrium ($X^2$=3.54, p=0.06). Seven participants failed to attend for recognition memory testing and were excluded from further analysis. Genotype frequencies of participants included in the analysis are given in Table 1. Demographic characteristics (age and IQ) are given in Table 2. IQ data were missing for two participants. Differences in demographic variables between genotype groups were assessed in a multivariate Analysis of Variance (ANOVA) with age and IQ as dependent variables and ADRA2B (del, no del) and COMT (val/val, val/met,

met/met) genotypes as between-subjects factors. There were no significant main effects of genotype and no interactions.

[Tables 1 and 2 about here]

3.2. Arousal and valence ratings

There were no differences between genetic variants in arousing *vs*. non arousing judgements made at encoding for COMT ($X^2$=1.7, p =0.42) or ADRA2B ($X^2$=1.9, p=0.16). The ratings made at recognition memory testing were significantly correlated with the standardised ratings for valence (r=0.72, p<0.0001) and arousal (r=0.63, p<0.0001). These ratings were entered into separate repeated measures ANOVA with emotional category (aversive, neutral) and stimulus category (encoding, foil) as the within-subject factors and ADRA2B (del, no del) and COMT (val/val, val/met, met/met) genotypes as between-subjects factors. There was a main effect of emotional category on both arousal [F(1, 91)=424.96, p < 0.001] and valence [F(1, 91)=897.6, p < 0.001] with emotional pictures being rated as significantly more arousing [mean(SD) = 6.53(1.08) *vs*. 4.03(1.04)] and more unpleasant [mean(SD) = 3.01(0.94) *vs*. 5.53(0.64)] than neutral. There were no main effects of genotype or stimulus category and no interactions.

3.2. Emotional Memory

R and d' were entered into separate repeated measures Analysis of Variance (ANOVA) with emotional category (aversive, neutral) as the within-subject factor and ADRA2B (del, no del) and COMT (val/val, val/met, met/met) genotypes as between-subjects factors. This revealed a significant emotion x COMT interaction [F(2, 90) = 3.4, p = 0.04] on recollection that

accounted for 7% of the variance in the model, see Figure 2. There were no other main effects or interactions. Post hoc independent t-tests indicated that recollection estimates were significantly greater in COMT val/val [t(57)=2.52, p=0.01] and val/met [t(70)=2.60, p=0.01] individuals compared to met/met for aversive, but not neutral stimuli. ANOVA did not reveal any main effects or interactions in relation to familiarity. Mean hit/false alarm rates, confidence ratings, recollection/familiarity estimates are given in Tables 3, 4, and 5 respectively. The average ROC curves for the aversive and neutral conditions for the COMT val/val and met/met genotypes are presented in Figure 3a-d.

[Tables 3, 4 and 5 and Figure 3 about here please]

We have previously reported analyses of the standard SDT parameters sensitivity (d') and response bias (C), in which we found no effect of COMT on emotional enhancement of memory (Gibbs et al., 2010b). However in the present analysis, we additionally examined d' based on hit rate and false alarm rate parameters derived only from responses in the highest confidence category. This produced a significant interaction between COMT and ADRA2B on overall memory performance, as previously reported, but no other significant main effects or interactions. However, in the model including only high confidence responses, the interaction between COMT genotype and arousal explained a greater proportion of the variance (3%) than the model including all responses (1%).

## *4. Discussion*

The aim of this study was to explore the contribution of genetic variation to the recollection and familiarity components of emotional enhancement of memory and to the best of our knowledge, this is the first study to do so. Given that previous studies have indicated that the

catecholamine neurotransmitters dopamine and noradrenaline play a key role in emotional processing, we examined the influence of two functional polymorphisms involved in catecholamine signalling (COMT val158met and the ADRA2B deletion polymorphism) on a dual process signal detection (DPSD) model of emotional memory. We found a significant interaction effect between COMT val158met genotype and emotional arousal on the recollection component of recognition memory. Specifically, long-term recollection for emotionally arousing, but not neutral stimuli was impaired in met/met individuals, compared to val/val and val/met individuals. Although this interaction explained only a small proportion of the variance in the model, the observed increases in effect size when using DPSD measures *vs*. standard signal detection theory (SDT) measures based on high-confidence responses *vs.* standard SDT measures based on all responses further supports the validity of the DPSD approach in this case. No prior studies have directly investigated the potential link between the COMT val158met polymorphism and emotional memory. The present findings, along with our previous report constitute the first published attempts to do so. Our present data suggest COMT moderation of high confidence recollective emotional memory processes, but not low confidence familiarity-based ones. This may be attributable to one of two possibilities: (i) a specific effect of COMT genotype on recollection, but not familiarity or (ii) greater power to detect effects on recollection given that emotional arousal is known to boost recollection to a greater degree than familiarity (Ochsner, 2000;Sharot et al., 2007;Gibbs et al., 2010a). The increasing effect sizes when moving from analysis of decisions made with any level of confidence levels to high confidence decisions to recollection, supports the latter scenario. This suggests that future studies aiming to investigate effects of COMT genotype on emotional modulation of recognition memory may maximise detection of statistically significant results by limiting analyses to responses made with the highest degree of confidence, or adopting a DPSD approach.

Prior to this, no studies had specifically examined the role of COMT in declarative emotional memory. In the absence of an existing body of behavioural emotional memory literature, it is difficult to interpret our findings in the context of specific differential effects of the val *vs.* met alleles. Previous studies investigating links between COMT and emotional processing have focussed on neural (as opposed to behavioural) effects and found that carriers of the met allele showed greater prefrontal cortical and amygdala activation in response to aversive scenes compared to carriers of the val allele (Domschke et al., 2008;Drabant et al., 2006;Smolka et al., 2005;Yacubian et al., 2007). It has therefore been suggested that the effect of COMT on emotional memory might relate to prefrontal regulation of amygdala reactivity to aversive events, in turn influencing consolidation of such events (Todd et al., 2011). This would be in keeping with the established role of the amygdala in modulating emotional memory (Cahill et al., 1996;Canli et al., 1999;Canli et al., 2000). However these studies clearly document positive correlations between amygdala activation and emotional enhancement of memory, suggesting that the increased activation amygdala activation observed in met carriers would predict enhanced, rather than impaired recollection for aversive stimuli as we observed. However, the relationship between the magnitude of brain activation as detected by functional neuroimaging and task performance remains complex and poorly understood. For example, some brain regions such as the prefrontal cortex appear to demonstrate a distinctive 'inverted U' or 'capacity-constrained' pattern of brain activation in relation to performance, such that the two are positively correlated up to the point that a capacity is reached, after which performance typically declines, and is uncoupled from brain activation (Callicott et al., 1999). Whilst this principle has not been specifically investigated in relation to the amygdala and other limbic structures involved in emotional memory, a similar pattern may also be applicable. For example, above a certain threshold, amygdala

activation may cease to enhance memory performance and begin to result in impairment. This would explain our present findings and would be consistent with other work supporting an inverted-U shaped relationship between corticosteroids and cognition, including episodic memory performance (Roozendaal, 2000;Lupien and McEwen, 1997;Wolkowitz et al., 1990). However, functional neuroimaging studies examining the relationship between COMT genotypes and brain activation associated with successful encoding of emotional stimuli will be necessary to clarify the neural basis of these genotype effects on emotional memory.

Three prior studies have investigated ADRA2B influences on emotional memory. In a large single-gene study, de Quervain and colleagues examined 435 Swiss participants and found that carriers of the deletion variant showed significantly greater recall memory for emotional (positive and negative) *vs*. neutral pictures compared to non carriers (de Quervain et al., 2007). In a further study to examine the relationship between genotype-dependent differences in brain activation and emotional memory, they collected fMRI from 57 participants using the same emotional memory task (Rasch et al., 2009). Deletion carriers demonstrated significantly increased amygdala activation in response to emotionally arousing pictures compared to non carriers, although no behavioural differences in emotional memory were observed. This was considered due to low statistical power (32%) to detect the effect size of 0.4 in this much smaller sample. In an independent study examining both COMT and ADRA2B genotype influences on emotional memory in 97 British participants using aversive emotional pictures, we did not find any ADRA2B-related differences in emotional memory for aversive compared to neutral pictures based on standard SDT parameters (Gibbs et al., 2010b). In the present analysis of these data using a DPSD approach, we also did not find any main effect of the ADRA2B genotype on emotional enhancement of recollection or familiarity processes. In both cases this may be due to insufficient statistical power (60%)

based on the effect size reported in the Swiss study. However, other methodological differences may also be relevant. Firstly, we used a 1 week interval between encoding and memory testing as opposed to their short 10 minute retention interval. It is possible that the genotype-dependent differences observed by de Quervain and colleagues relate to encoding processes, such as attention and perception, influencing short-term memory, as opposed to the consolidation processes contributing to long-term memory. This would be consistent with their fMRI findings where ADRA2B genotype-dependent differences in amygdala activation were observed during encoding but did not influence subsequent memory. However, given that memory was tested at a single time point in both studies, it remains unclear whether the effects are due to encoding or consolidation processes, or both. Further studies will be necessary to clarify the effects of ADRA2B on the affective modulation of attention/perception during encoding and the effects of post-encoding arousal (Todd et al., 2011). Although no genotype effects on emotional memory were found in our prior study, we did observe a significant interaction between COMT and ADRA2B on recognition memory for both aversive and neutral pictures, such that possession of the ADRA2B deletion appeared to ameliorate the impairment associated with the COMT val allele (Gibbs et al., 2010b). Yet no such interaction was observed in relation to recollection and familiarity in the present analysis. This may be because the epistatic effects of the two genes on overall recognition memory may influence both recollection and familiarity and examining these processes separately may have resulted in a loss of power. Further studies are warranted using larger samples.

In addition to issues that may have arisen due to low statistical power, there are a number of limitations to our study. The COMT genotypes included in the final analysis deviated from HWE, raising the possibility of genotyping error or a selection bias limiting the

generalisability of our findings. However, we consider this unlikely given that both genotypes were in HWE in the full sample of recruited individuals, although we acknowledge that the $X^2$ analysis for COMT was suggestive of trend-level statistical significance. Additionally, we used an all male group due in order to avoid confounding the established gender differences in emotional memory and its neural correlates (Cahill, 2006) as well as sexually dimorphic effects of COMT (Chen et al., 2004;Harrison and Tunbridge, 2007;Gogos et al., 1998). Therefore, the extent to which the present findings are applicable to women remains unclear and will warrant further investigation in larger mixed-gender samples to allow adequate control for possible gender effects. Furthermore, we used only aversive emotionally arousing pictures. This is justified by the accumulating evidence that COMT preferentially influences processing of aversive as opposed to positive emotional stimuli (Herrmann et al., 2009;Montag et al., 2008;Smolka et al., 2007) and the fact that emotional enhancement of memory is arousal-dependent, rather than valence-dependent (Kensinger and Corkin, 2004). Nevertheless, the inclusion of only aversive emotional stimuli means that implications of our findings for positive emotional experiences are unclear. Finally, we examined only two genes amongst a number of variants that may contribute to emotional memory and its components (Todd et al., 2011). It is increasingly understood that epistatic effects are likely to be at least as important in modulating behavioural phenotypes, if not more so, than single-gene effects (Elvevåg and Weinberger, 2009). In fact, it has been suggested that one of the reasons that studies of single polymorphisms replicate poorly across independent samples is because epistasis is more important (Moore and Williams, 2002). In spite of this, gene-gene interactions have rarely been explored in behavioural genetics studies. This is because the phenomenon of biological epistasis is poorly understood, its analysis is more complex and as the number of genes involved increases, exponentially larger sample sizes are needed to

estimate interaction effects (Moore, 2008). Indeed, the small n for each genotype combination in our present study may have limited power to detect such an effect.

In summary, the present study provides preliminary behavioural evidence concerning the effect of the COMT val158met polymorphism on the recollection component of emotional memory. As such, it contributes to an important emerging area of cognitive neuroscience research: the role of genetic variation in emotional enhancement of memory. It also begins to disentangle the effects in relation to two different forms of episodic memory: recollection and familiarity. However, it is acknowledged that the findings reported are preliminary and future studies are needed to independently replicate and extend this work.

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**Tables**

Table 1 – Genotype Frequencies

| *Genotype* | | | *COMT* [2] | | *Total* |
|---|---|---|---|---|---|
| *ADRA2B* [1] | | val/val | val/met | met/met | |
| del | del/del | 1 | 3 | 4 | 8 |
| | del/no del | 16 | 17 | 15 | 48 |
| no del | no del/no del | 8 | 17 | 16 | 41 |
| | *Total* | 25 | 37 | 35 | 97 |

[1] $X^2 = 1.38$, $p = 0.24$ [2] $X^2 = 5.08$, $p = 0.02$

Table 2 – Socio-demographic characteristics for COMT and ADRA2B genotypes

| | *COMT* | | | | | | *ADRA2B* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | val/val | val/met | met/met | *F* | *df* | *p* | del | no del | *F* | *df* | *p* |
| Age [yrs, mean (SD)] | 24.7 (5.0) | 24.1 (5.0) | 23.8 (4.6) | 0.89 | 2, 90 | 0.12 | 24.8 (5.1) | 23.2 (4.3) | 3.04 | 1, 90 | 0.08 |
| IQ [mean (SD)] | 106.4 (7.5) | 105.0 (5.6) | 106.2 (5.4) | 0.50 | 2, 90 | 0.63 | 106.7 (5.7) | 104.7 (6.4) | 3.43 | 1, 90 | 0.07 |

Table 3 – Mean (SD) hit rate and false alarm rate for aversive and neutral stimuli for COMT and ADRA2B genotypes

| | | | *Hit Rate* | | *False Alarm Rate* | |
|---|---|---|---|---|---|---|
| *COMT* | *ADRA2B* | N | Aversive | Neutral | Aversive | Neutral |
| *val/val* | *no del* | 8 | 0.70(0.23) | 0.55(0.21) | 0.25(0.13) | 0.21(0.13) |
| | *del* | 17 | 0.81(0.10) | 0.69(0.14) | 0.21(0.13) | 0.14(0.08) |
| *Total* | | 25 | 0.77(0.16) | 0.65(0.17) | 0.22(0.13) | 0.16(0.10) |
| *val/met* | *no del* | 17 | 0.80(0.12) | 0.69(0.12) | 0.22(0.15) | 0.15(0.14) |
| | *del* | 20 | 0.80(0.11) | 0.63(0.14) | 0.19(0.10) | 0.11(0.07) |
| *Total* | | 37 | 0.80(0.11) | 0.66(0.13) | 0.20(0.13) | 0.13(0.11) |
| *met/met* | *no del* | 16 | 0.82(0.02) | 0.69(0.12) | 0.18(0.07) | 0.13(0.07) |
| | *del* | 19 | 0.76(0.13) | 0.62(0.14) | 0.19(0.08) | 0.15(0.10) |
| *Total* | | 35 | 0.79(0.11) | 0.65(0.14) | 0.18(0.08) | 0.14(0.08) |

Table 4 – Mean (SD) confidence ratings for hits and false alarms for aversive and neutral stimuli for COMT and ADRA2B genotypes

| | | | *Hits* | | *False Alarms* | |
|---|---|---|---|---|---|---|
| *COMT* | *ADRA2B* | N | Aversive | Neutral | Aversive | Neutral |
| *val/val* | *no del* | 8 | 7.33(1.10) | 6.93(1.22) | 6.25(1.48) | 5.40(1.80) |
| | *del* | 17 | 7.76(0.72) | 6.28(1.56) | 6.28(1.56) | 4.86(1.33) |
| *Total* | | 25 | 7.62(0.86) | 7.13(0.92) | 6.27(1.40) | 5.03(1.48) |
| *val/met* | *no del* | 17 | 7.72(0.53) | 7.28(0.82) | 6.01(1.43) | 5.18(1.35) |
| | *del* | 20 | 7.52(0.61) | 7.20(0.82) | 5.73(1.30) | 5.23(1.38) |
| *Total* | | 37 | 7.61(0.58) | 7.23(0.81) | 5.86(1.53) | 5.21(1.35) |
| *met/met* | *no del* | 16 | 7.87(0.66) | 7.49(0.64) | 6.43(0.90) | 5.59(1.21) |
| | *del* | 19 | 7.41(0.87) | 6.73(1.19) | 5.75(1.40) | 4.31(1.66) |
| *Total* | | 35 | 7.62(0.80) | 7.08(1.04) | 6.06(1.23) | 4.90(1.59) |

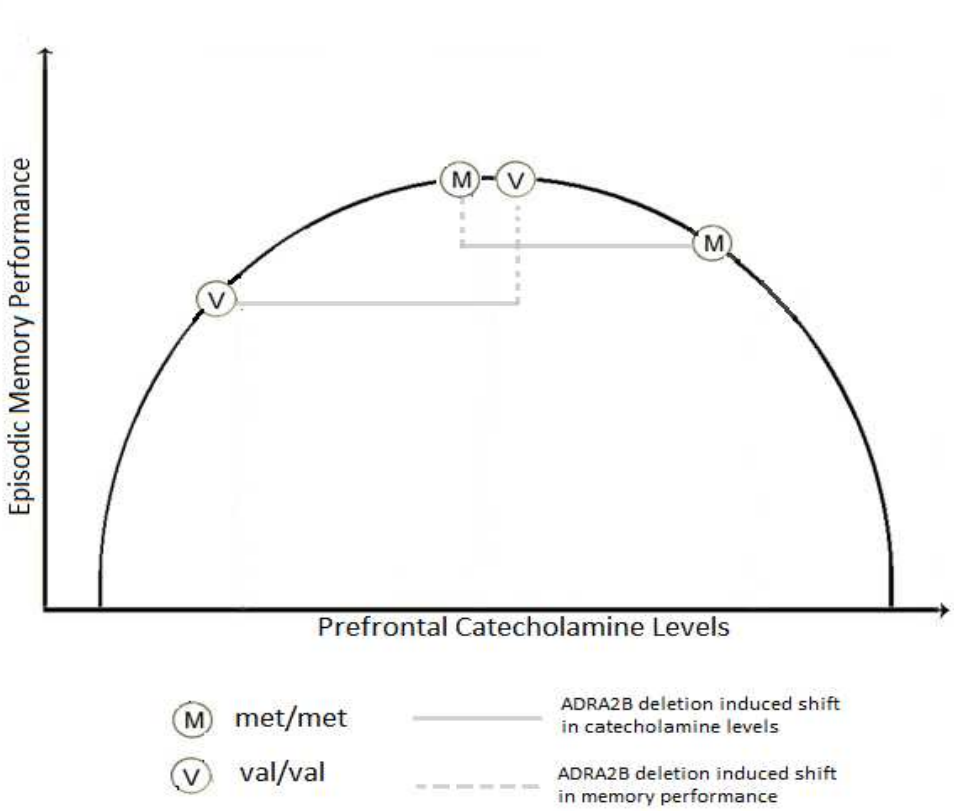
Table 5 – Mean (SD) recollection and familiarity estimates for aversive and neutral stimuli for COMT and ADRA2B genotypes

| | | | *Recollection* | | *Familiarity* | |
|---|---|---|---|---|---|---|
| *COMT* | *ADRA2B* | N | Aversive | Neutral | Aversive | Neutral |
| *val/val* | *no del* | 7 | 0.29(0.32) | 0.15(0.14) | 1.33(0.96) | 0.76(0.46) |
| | *del* | 17 | 0.41(0.28) | 0.35(0.25) | 1.31(0.71) | 1.08(0.53) |
| *Total* | | 24 | 0.38(0.29) | 0.29(0.24) | 1.32(0.77) | 0.99(0.52) |
| *val/met* | *no del* | 17 | 0.35(0.30) | 0.30(0.20) | 1.17(0.81) | 1.10(0.53) |
| | *del* | 20 | 0.37(0.25) | 0.26(0.18) | 1.30(0.68) | 1.30(0.52) |
| *Total* | | 37 | 0.36(0.27) | 0.28(0.19) | 1.24(0.74) | 1.21(0.53) |
| *met/met* | *no del* | 16 | 0.22(0.30) | 0.25(0.19) | 1.59(0.55) | 1.33(0.59) |
| | *del* | 19 | 0.18(0.23) | 0.27(0.16) | 1.33(0.70) | 1.07(0.41) |
| *Total* | | 35 | 0.20(0.26) | 0.26(0.17) | 1.45(0.64) | 1.19(0.51) |

**Figures**

Figure 1

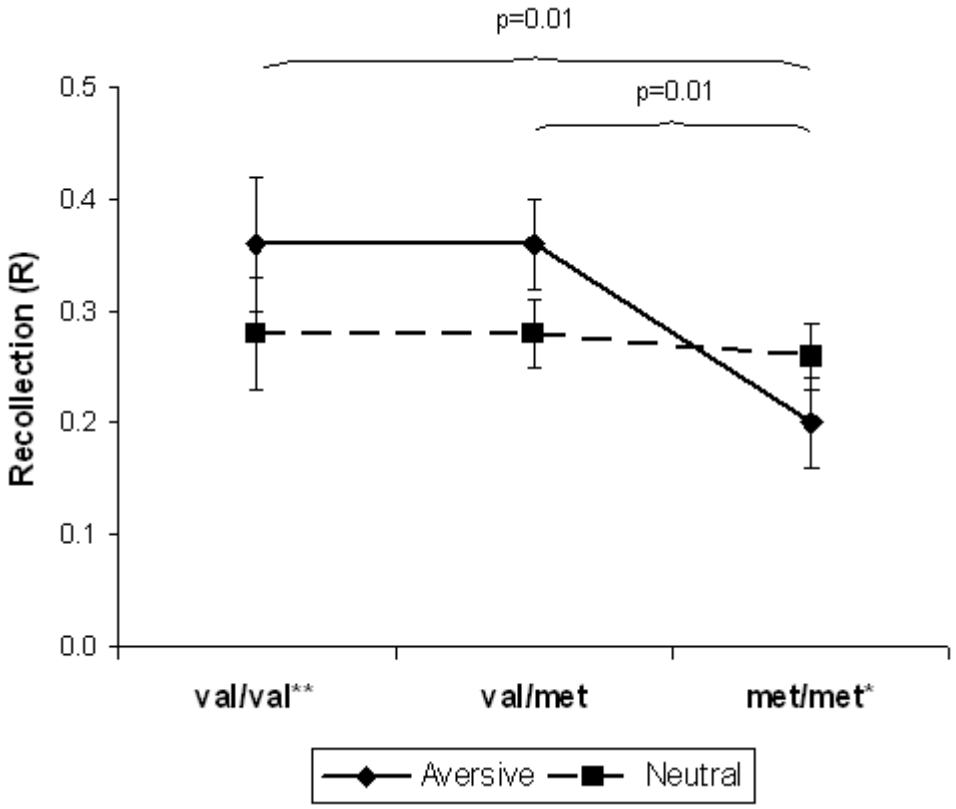
Title: Inverted "U" shaped model of episodic memory performance against catecholamine levels as influenced by genotype.




Description: A putative inverted "U" models the effects of COMT and ADRA2B genotypes on episodic memory performance and catecholamine levels in the prefrontal cortex (PFC). By presumably boosting noradrenergic catecholamine transmission, the deletion variant of ADRA2B shifts COMT val/val individuals - who normally have greater COMT activity, less dopaminergic catecholamine transmission, and relatively poorer episodic memory - to the right, and more optimal performance. The ADRA2B deletion variant also shifts met/met individuals, who are presumed to have optimal prefrontal function, to the right, but to less efficient performance.

Figure 2

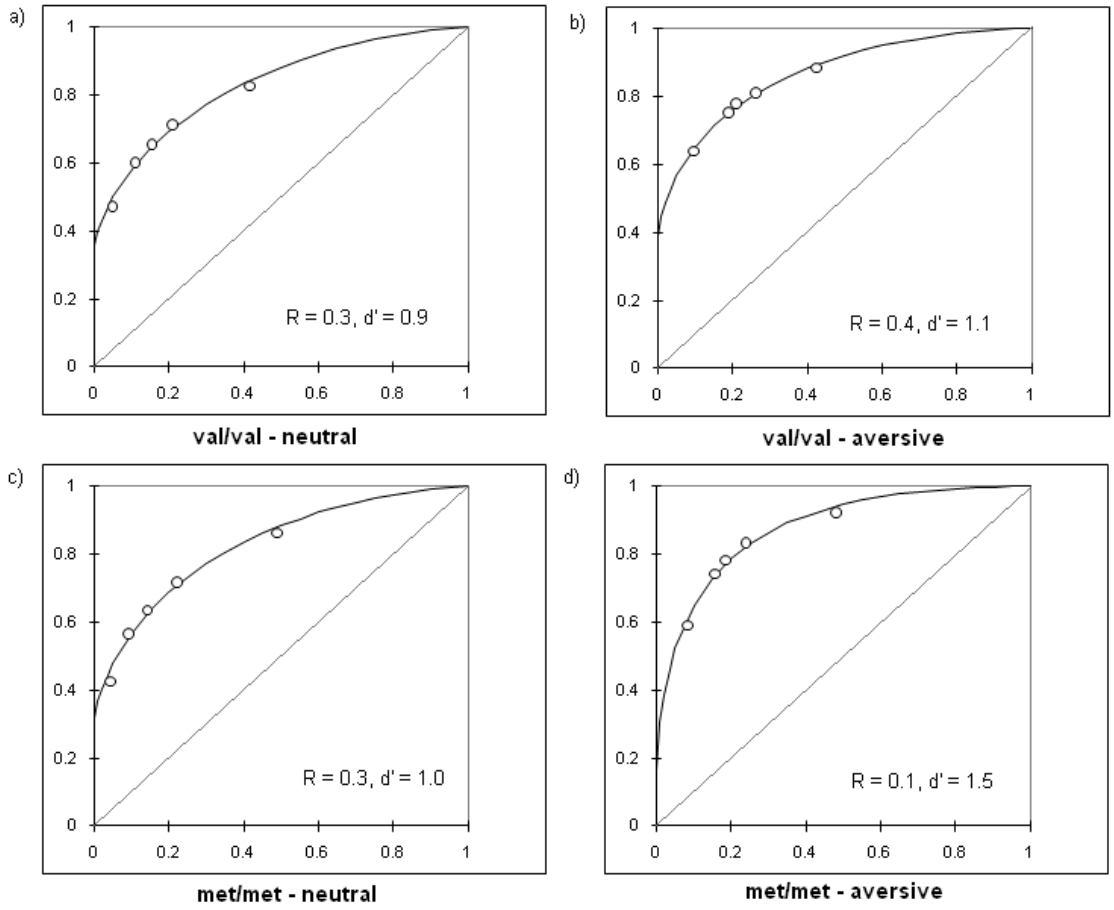
Title: Recollection estimates in COMT genotypes for aversive and neutral stimuli




Description: There was a significant interaction between COMT genotype and stimulus valence on recollection. Recollection estimates were significantly lower in COMT met/met genotypes compared to val/val and val/met for aversive, but not neutral stimuli.

Figure 3

Title: ROC curves for COMT val/val and met/met genotypes for aversive and neutral conditions plotted in probability space




Description: The x-axis represents the proportion of new pictures incorrectly identified as "old". The y-axis represents the proportion of old pictures correctly identified. The diagonal represents chance discrimination. Functions a to c are curvilinear and asymmetrical along the diagonal, suggesting contributions of both recollection and familiarity to the recognition memory process (Yonelinas et al, 1998). Function b is skewed relative to a, suggesting a greater contribution of recollection for the aversive pictures, compared to neutral pictures in the val/val genotypes. Function d appears symmetrical along the diagonal, rather than skewed relative to c, suggesting that recollection made a limited contribution to the discrimination of aversive pictures, compared to neutral pictures in the met/met genotypes.

# Article 3. Epistasis between 5-HTTLPR and ADRA2B polymorphisms influences attentional bias for emotional information in healthy volunteers

**Short title:** Epistasis between 5-HTTLPR and ADRA2B

**Category:** Regular research article

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## *Abstract*

Individual differences in emotional processing are likely to contribute to vulnerability and resilience to emotional disorders such as depression and anxiety. Genetic variation is known to contribute to these differences but they remain incompletely understood. The serotonin transporter (5-HTTLPR) and alpha 2B adrenergic autoreceptor (ADRA2B) insertion/deletion polymorphisms impact on two separate but interacting monaminergic signalling mechanisms that have been implicated in both emotional processing and emotional disorders. Recent studies suggest that the 5-HTTLPR *s* allele is associated with a negative attentional bias and an increased risk of emotional disorders. However such complex behavioural traits are likely to exhibit polygenicity, including epistasis. This study examined the contribution of the 5-HTTLPR and ADRA2B insertion/deletion polymorphisms to attentional biases for aversive information in 94 healthy male volunteers and found evidence of a significant epistatic effect ($p<0.001$). Specifically, in the presence of the 5-HTTLPR *s* allele, the attentional bias for aversive information was attenuated by possession of the ADRA2B deletion variant whereas in the absence of the *s* allele, the bias was enhanced. These data identify a cognitive mechanism linking genotype-dependent serotonergic and noradrenergic signalling that is likely to have implications for the development of cognitive markers for depression/anxiety as well as therapeutic drug effects and personalised approaches to treatment.

**Keywords:** 5-HTTLPR; ADRA2B; emotional processing

## *Introduction*

Enhanced processing of emotionally salient relative to neutral information is normally considered to be an adaptive process enabling threat detection and increasing the probability of survival (Vuilleumier, 2005). However, there is considerable evidence that biased processing of emotional information also plays a role in the aetiology and maintenance of emotional disorders such as depression and anxiety (Leppanen, 2006). The monoamine neurotransmitters (serotonin, dopamine and noradrenaline) are known to play a significant role in emotional processing and although they are generally considered to act synergistically, few studies have specifically investigated interactions between these neurotransmitters.

Serotonin and noradrenaline are also heavily implicated in the aetiology of emotional disorders and the majority of therapeutic agents increase synaptic levels of these neurotransmitters (Nutt, 2002). Despite their use for over half a century, it remains unclear how antidepressants exert their therapeutic effects. More recently, evidence has emerged that suggesting that serotonergic and noradrenergic antidepressant drugs may act by modifying emotional processing biases (Harmer *et al.* 2009). Yet in spite of this increasing insight, the fact that up to 50% of patients treated with these medications fail to respond adequately to remains a significant challenge in the management of these disorders (Souery *et al.* 1999). Inter-individual differences in responses to emotional stimuli may contribute to differences in vulnerability to emotional disorders as well as response to therapeutic agents (Hamann and Canli, 2004). It is increasingly accepted that genetic factors explain small but significant amounts of this variability (Todd *et al.* 2011). Thus, polymorphisms in genes involved serotonergic and noradrenergic signalling represent apposite candidates for further investigation.

One of the genetic variants that have been most extensively investigated in relation to human emotional processing and emotional disorders is the gene encoding the serotonin transporter (5-HTT or SLC6A4). An insertion/deletion polymorphism in the promoter region of this gene (5-HTTLPR) results in 2 common allelic variants: short (*s*) and long (*l*). The former has been associated with reduced transporter transcription, resulting in approximately 50% reduction in transporter availability in vitro and presumed increased synaptic serotonin availability (Heils *et al.* 1996). More recently, an additional A/G SNP in the *l* allele (rs25531) has been found to further influence transcriptional activity. The G variant of the *l* allele is considered to result in a reduction in transcriptional efficiency to a level similar to that of the *s* allele (Hu *et al.* 2005; Wendland *et al.* 2006). The frequency of this G allele varies with ethnicity however it is relatively uncommon in white European ethnic groups (Hu *et al.* 2006). Early seminal studies linked the *s* allele to increased neurotic personality traits (Lesch *et al.* 1996) and an increased risk of depression in the context of adverse life events (Caspi *et al.* 2003). Subsequent studies examining the in vivo effects of this genetic variation on the phenotypic expression of the serotonin transporter in the human brain produced inconsistent results (Praschak-Rieder *et al.* 2007; Reimold *et al.* 2007; van Dyck *et al.* 2004; Willeit *et al.* 2000). However, consistent with a number of prior studies, a relatively large recent positron emission tomography (PET) study in healthy volunteers found that polymorphic variation in 5-HTTLPR did not alter expression of the serotonin transporter (Murthy *et al.* 2010). It has however been suggested that this genetic variation instead contributes to early neurodevelopmental changes that may impact on brain structure and function in later life (Lesch and Gutknecht, 2005). This would be consistent with the further body of functional magnetic resonance imaging (fMRI) literature that has more consistently documented that *s* allele carriers demonstrate significantly greater amygdala activation in response to aversive, relative to neutral, stimuli in a variety of emotional processing tasks (Bertolino *et al.* 2005;

Canli *et al.* 2005; Hariri *et al.* 2002; Hariri *et al.* 2005; Heinz *et al.* 2004; Pezawas *et al.* 2005); for a meta-analysis see (Munafò *et al.* 2008). Yet, the behavioural implications of these neural differences remained unclear. More recently a number of studies have focused on 'behavioural endophenotypes' such as selective attentional biases for emotional information. To date, these studies have demonstrated an association between the 5-HTTLPR *s* allele and preferential attention to aversive stimuli (Beevers *et al.* 2007; Beevers *et al.* 2010; Beevers *et al.* 2011; Fox *et al.* 2009; Osinsky *et al.* 2008). However, these reports have not been entirely consistent (Caspi *et al.* 2010). One important potential source of inconsistency and non replication in genetics studies of complex quantitative traits is the issue of polygenicity, including biologic epistasis (Moore, 2008). Yet none of these neuroimaging or behavioural genetics studies has examined the effects of the other major neurotransmitter system implicated in affective spectrum disorders and emotional processing: the noradrenergic system.

Noradrenaline has an established role in modulating memory enhancement for emotionally arousing information (McGaugh, 2004) and recent pharmacological challenge studies indicate that it is also involved in modulating attentional biases for emotional information in healthy human volunteers (De Martino *et al.* 2008). However the contribution of genetically influenced differences in noradrenergic tone to inter-individual differences in human emotional processing has been largely unexplored. An insertion/deletion polymorphism in the alpha 2b adrenergic (auto)receptor gene (ADRA2B) has recently been found to contribute to individual differences in emotionally influenced memory processes. The deletion variant (Del301-303) is associated with decreased agonist-promoted phosphorylation and receptor desensitisation *in vitro* (Small *et al.* 2001), presumed to be associated with increased noradrenergic tone *in vivo*. In two seminal studies, de Quervain and colleagues demonstrated

an association between this polymorphic variant, increased amygdala reactivity and an increased memory bias for emotional stimuli (de Quervain *et al.* 2007; Rasch *et al.* 2009). However, the contribution of ADRA2B to emotionally enhanced attentional processes was not explored. It therefore remains possible that the observed memory bias arises due to an attentional advantage contributing to enhanced encoding of emotional information (Todd *et al.* 2011). The purpose of this study was therefore to test the hypothesis that an increased attentional bias for emotionally arousing information is associated with the deletion variants of ADRA2B and 5-HTTLPR and examine whether these effects are subject to additive or non-additive interactions.

## *Materials and methods*

*Participants*

One hundred and seven healthy white British male volunteers between the ages of 18 and 35 (mean 24.0 ± 4.8) were recruited from the university and local community. They had no lifetime history of psychiatric or neurological disorder. Estimates of verbal IQ were derived from the National Adult Reading Test (NART) (Nelson, 1982). The study was approved by the local research ethics committee. Following complete description of the study to the participants, written informed consent was obtained.

*Behavioural task*

We used an emotional attention blink (AB) task based on dual-target rapid serial visual presentation (RSVP) methodology (Raymond *et al.* 1992). Identification of a 1$^{st}$ target (T1) in a rapid stream of stimuli leads to transient impairment in identification of a 2$^{nd}$ target (T2) – an effect, known as the *attentional blink*. It has previously been used by ourselves and

others to demonstrate a bias towards accurate detection of aversive T2 targets compared to neutral (Anderson, 2005; Anderson and Phelps, 2001; De Martino *et al.* 2008; Gibbs *et al.* 2007; Keil and Ihssen, 2004). The task comprised 168 trials, each trial consisting of 13 white distracter words and 2 green target words (T1 and T2) presented sequentially in the centre of a laptop computer screen; see Figure 1. T1 stimuli were all neutral words averaging 4.8 letters in length. T2 words were derived from the Affective Norms for English Words (Bradley and Lawson, 1999) and half were aversive-arousing (mean valence and arousal ratings of 2.5 and 7.0, respectively) and half were neutral (mean valence and arousal ratings of 5.1 and 3.5, respectively). Aversive and neutral T2 stimuli did not differ significantly in letter length (mean = 5.1 vs 4.8, respectively; p=0.21) or written word frequency (mean = 67.1 vs 87.9, respectively; p=0.50) (Kucera and Francis, 1967). Distracter items were 92 words of longer length (mean letters = 12.5) to facilitate masking of the targets. Each item was presented for 100 milliseconds (ms) and was immediately followed by the subsequent item. The lag between the T1 and T2 targets was varied to contain one, three, or five intervening distracters (Lag 2, Lag 4 or Lag 6) with corresponding Stimulus Onset Asynchronies (SOAs) of 200ms, 400ms or 600ms. Participants were instructed to ignore the words in white (distracters) and identify the two green target words (T1 and T2). Responses were made by participants writing down the two targets in any order immediately after each trial on sheets that were subsequently scored. Exactly correct spelling was not necessary for a correct response. Vowel and consonant omissions, insertions or replacements were allowed provided the word was recognisable and the spelling was phonologically accurate.

Figure 1 about here

*Genotyping*

DNA extraction and genotyping for the ADRA2B insertion/deletion polymorphism was carried out by KBioscience, Hertfordshire, UK as previously reported (Gibbs *et al.* 2010). For the 5-HTTLPR insertion/deletion, polymerase chain reaction (PCR) was also carried out using KBioscience in-house single nucleotide polymorphism (SNP) genotyping system (KASPar®) using fluorescently labelled primers (pF1:Cy5.5-CCCAGCGTGCTCCAGAAAC;
pR:GGACCTGGGCAGTTGTGC). For technical reasons, we were unable to complete further triallelic genotyping of the A/G SNP (rs25531) in the 5-HTTLPR insertion allele.

*Statistical analysis*

Hardy-Weinberg equilibrium (HWE) of genetic data was assessed by $\chi^2$ analysis. Possible genotype-dependent differences in demographic variables between genotype groups were assessed in a multivariate analysis of variance (ANOVA) with age and IQ as dependent variables and 5-HTTLPR and ADRA2B genotypes as between-subjects factors. Genotype effects on T1 detection in the AB task were examined in a univariate analysis of variance with percent correct T1 report as the dependent measure and 5-HTTLPR and ADRA2B genotypes as the between-subjects factors. Affective modulation of the AB effect was examined in a repeated measures ANOVA with the same between-subjects factors, valence (aversive, neutral) and lag (2, 4 and 6) as within-subject factors and percent correct T2 report [contingent on the correct identification of T1][4] as the dependent measure. Significant interactions were explored using post hoc t-tests. A Greenhouse-Geisser correction was applied where sphericity assumptions were violated.

[4] This is to guarantee that proper attention has been devoted to T1 to ensure an AB effect.

## *Results*

*Genotypes*

Of the 107 participants, ADRA2B genotypes were unavailable for 2 participants, 11 were homozygous carriers of the ADRA2B deletion, 48 were heterozygotes and 46 were non carriers, consistent with HWE ($\chi^2$=0.09, p=0.77). Due to the small number of homozygous carriers, they were combined with the heterozygotes, giving two genotype groups of deletion carriers (Del) and non carriers (Ins) as previously done by ourselves and others (de Quervain *et al.* 2007; Gibbs *et al.* 2010). For 5-HTTLPR, 12 genotypes were unavailable, 35 were homozygous *l*/*l*, 41 were heterozygous *s*/*l* and 19 were homozygous *s*/*s*, consistent with HWE ($\chi^2$=1.18, p=0.28). Given that the *s* allele is considered to have a dominant effect (Lesch *et al.* 1996), participants were divided into two groups: homozygous or heterozygous *s* allele carriers (S group) and non carriers (L group) consistent with prior studies (Canli *et al.* 2005). Participants for whom genetic data were not available for both polymorphisms were excluded from further analysis, leaving a total sample of 94. Demographic characteristics (age and IQ) are given in Table 1. There were no genotype effects on these variables.

Table 1 about here

*Behavioural data*

There was a significant main effect of valence on T2 detection accuracy [F(1, 90) = 11.0, p = 0.001], with greater detection of aversive (92.7% ± 1.6) compared to neutral (81.2% ± 1.2) words. T2 detection accuracy also increased significantly [F(1.2, 109.4) = 183.3, p < 0.001] as the temporal lag between the targets increased [lag 2 = 64.4% ± 22.2; lag 4 = 83.8% ± 15.7, lag 6 = 91.1% ± 12.3] and there was a significant lag x valence interaction [F(2, 180 = 28.5, p < 0.001] such that the emotional attentional bias was most pronounced at lag 4 (9%) compared to lag 6 (3%) and lag 2 (-3%) where it was absent. There were no main effects of

the individual genes however there was a highly significant ADRA2B x 5-HTTLPR x valence interaction [$F(1, 90) = 15.0$, $p < 0.001$]. In order to clarify this interaction we first conducted a separate repeated measures analysis of variance in the 5-HTTLPR S and L groups with T2 detection as the dependent variable, valence (aversive, neutral) as the between subjects variable and ADRA2B genotype as the between subjects variable. We found significant ADRA2B x valence interactions in both 5-HTLPR S [$F(1, 58) = 8.2$, $p = 0.006$] and L [$F(1, 32) = 6.06$, $p = 0.02$] groups. Post hoc paired t-tests demonstrated that in the 5-HTTLPR L group, there was a significant attentional bias for aversive vs. neutral T2 words in ADRA2B deletion carriers [$t(20) = 3.0$, $p = 0.007$, $d = 0.7$] that was absent in non carriers [$t(12) = -0.68$, $p = 0.504$]. Conversely, in the 5-HTTLPR S group, the significant emotional attentional bias was absent in ADRA2B deletion carriers [$t(29) = 0.477$, $p = 0.637$] but present in non carriers [$t(29) = 4.9$, $p < 0.001$, $d = 1$]. See Figure 2. There were no other significant main genotype effects or interactions in relation to T1 or T2 detection.

Figure 2 about here

## *Discussion*

The purpose of this study was to examine the effects of serotonergic (5-HTTLPR) and noradrenergic (ADRA2B) genetic variants on attentional biases for aversive stimuli using an attentional blink (AB) paradigm. To our knowledge, this is the first study to examine the contribution of the ADRA2B insertion/deletion polymorphism to individual differences in emotional attentional biases and only the second study to explore the genetic basis of the emotional AB effect - cf., Munafò and colleagues previously found an association between 5-HTTLPR genotype, smoking status and detection of smoking-related stimuli in an AB task (Munafò *et al.* 2005). The significant novel finding from this study is that the affective

modulation of T2 detection is influenced by a non-additive (epistatic) interaction between the ADRA2B and 5-HTTLPR insertion/deletion polymorphisms.

Specifically, we found that a significant attentional bias for aversive compared to neutral information was present in individuals possessing at least one copy of the short (*s*) allele of 5-HTTLPR, but only if they did not carry the ADRA2B deletion. Conversely, the attentional bias for emotional information was only present in 5-HTTLPR long (*l*) allele homozygotes if they were ADRA2B deletion carriers. This suggests that in the presence of the 5-HTTLPR *s* allele which is a putative risk allele for depressive and anxiety disorders, the negative attentional bias is attenuated by the ADRA2B deletion variant whereas in the absence of the *s* allele, the bias is enhanced. Both of these effects may be related to adaptive processes. For instance, dependent on 5-HTTLPR genotype, the effect of the ADRA2B deletion variant may be to either exert a protective effect against affective spectrum disorders or facilitate enhanced detection of threat, in both cases contributing to increased probability of survival.

*Behavioural genetics implications*

We did not find a main effect of the serotonin transporter polymorphism on emotional attention as a number of prior studies have done (Beevers *et al.* 2007; Fox *et al.* 2009; Munafò *et al.* 2005; Osinsky *et al.* 2008). However, with the exception of Munafò et al 2005, all of these studies have used a variation of the dot probe task to evaluate emotional biases in selective attention, rather than the AB task. Although both of these tasks evaluate selective attention when cognitive resources are limited, the latter measures deployment of attention resources under temporal constrains whilst the former typically utilises spatial limitations. Notably, Munafò et al 2005 also used a alternate variant of the AB task that indexes attention by establishing whether the detection of a neutral T2 target is impaired a

preceding emotionally salient or neutral T1 target. It is possible that these task-related differences may account for the difference in findings. Yet the studies reporting positive associations using the dot probe task are not without inconsistencies. For example some have linked the *s* allele to biases *toward* aversive stimuli (Beevers *et al.* 2007; Osinsky *et al.* 2008) whilst others suggest that the *l* allele results in biases *away* from negative stimuli (Fox *et al.* 2009; Kwang *et al.* 2010). Other discrepancies include 5-HTTLPR associations found only with long (Osinsky *et al.* 2008) or short (Beevers *et al.* 2007) stimulus presentation durations. In spite of using shared dot probe methodology, there are still been significant differences between these studies in terms of subjects (healthy volunteers vs psychiatric patients; men vs women), stimuli (words vs spiders vs pictorial scenes) and duration of stimulus presentation (<500ms vs > 500ms). These differences highlight the need for task consistency in future studies in order to facilitate replication (NCI-NHGRI Working Group on Replication in Association Studies, 2007).

The effect of the ADRA2B insertion/deletion polymorphism on attentional biases for emotional information has not been previously investigated. However it has been suggested that it might contribute to the emotional memory bias observed in ADRA2B deletion carriers (Todd and Anderson, 2009). We did not find any main effect of ADRA2B on emotional attention in this study suggesting that the ADRA2B deletion variant does not independently bias attention towards emotional stimuli but may interact with other monaminergic gene systems to contribute to such bias.

*Behavioural pharmacogenetics implications*

A number of studies have begun to examine possible interactions between 5-HTTLPR polymorphisms, emotional attentional biases and the effects of serotonergic manipulation by

acute tryptophan depletion (ATD) but have thus far failed to produce consistent findings (Firk and Markus, 2009; Markus and De Raedt, 2011; Markus and Firk, 2009; Roiser *et al.* 2007). This may in part be due to the fact that ATD in healthy volunteers, independent of genotype, has not produced entirely consistent effects on emotional processing (Hayward *et al.* 2005; Murphy *et al.* 2002; Rubinsztein *et al.* 2001). No studies have as yet examined the possible contribution of genetic variation to the effects of serotonergic and noradrenergic drugs on emotional attention. However, a number of pharmacological studies have examined the effects of serotonergic and noradrenergic drugs on emotional processing in healthy subjects (Arce *et al.* 2008; Arnone *et al.* 2009; Brühl *et al.* 2009; Harmer *et al.* 2008; Harmer *et al.* 2003; Harmer *et al.* 2004; Murphy *et al.* 2009a; Norbury *et al.* 2007; Rawlings *et al.* 2010). This work may be relevant to understanding the present findings however it is difficult to make direct comparisons between genetically and pharmacologically mediated effects on emotional processing as highlighted in the *Clinical Implications* below. Additionally, only three of these studies have specifically examined attentional biases and have produced relatively inconsistent findings (Browning *et al.* 2007; De Martino *et al.* 2008; Murphy *et al.* 2009b).

Using a dot probe task, Browning et al 2007 found that the administration of a single dose of the selective serotonin reuptake inhibitor (SSRI) antidepressant citalopram to healthy volunteers resulted in an attentional bias towards positive words (Browning *et al.* 2007). This is consistent with some of the behavioural findings in relation to the 5-HTTLPR *l* allele described above. On an AB task, De Martino et al found that a single dose of the noradrenaline reuptake inhibitor (NRI) reboxetine boosted detection of emotionally arousing compared to neutral words in healthy volunteers (De Martino *et al.* 2008). Also on a dot probe task in healthy volunteers, Murphy et al 2009 found that repeated citalopram

administration reduced the attentional bias towards emotional faces, independently of valence, whilst reboxetine had no effect (Murphy *et al.* 2009b). These apparently conflicting findings could again be related to differences in methodology (dot probe vs. AB; words vs. faces; single vs. repeated dosing). In fact, there is evidence from both animal and human studies that acute and chronic citalopram administration may differentially influence emotional processing such that acute doses result in an intial increase in the processing of negative information that is attenuated with repeated dosing (Burghardt *et al.* 2004; Harmer *et al.* 2003; Harmer *et al.* 2004). However genotype-dependent drug effects may also contribute to these differences. For example, pharmacological enhancement or attenuation of emotional attentional biases may be less prominent in individuals with genotype-related emotional processing biases or in whom such biases are absent, respectively. If emotional attentional bias is to function as an effective cognitive marker, future pharmacological challenge studies will also need to consider the contribution of genetic variations in the neurotransmitter systems under investigation. There is also an increasing need to evaluate the effects of genetic epistasis between these systems as this may have important clinical implications for the pharmacogenetics of depression and anxiety.

*Clinical implications*

That such biological epistasis exists between serotonergic and noradrenergic genes, is consistent with the fact that these neurotransmitter systems are intimately connected in the central nervous system (de Boer, 1995). Noradrenergic neurotransmission is modulated by presynaptic inhibitory alpha 2 adrenergic (auto)receptors and their blockade increases synaptic levels of noradrenaline. However there is evidence that serotonergic neurotransmission is also modulated by presynaptic alpha 2 adrenergic (hetero)receptors (Clement *et al.* 1992; De Boer *et al.* 1994; Mongeau *et al.* 1993). Yet precisely how these

systems may interact to produce the intermediate phenotypes and the clinical disorders themselves remains unclear. The fact that the majority of drugs used to treat affective spectrum disorders act by inhibiting the serotonin transporter seems at odds with the fact that individuals with genetically influenced reductions in serotonin transporter function have greater risks of developing these disorders, as well as poorer treatment response rates (Lesch and Gutknecht, 2005). This ostensible contradiction is increasingly understood in terms of the complex autoregulatory processes governing serotonergic function (Routledge and Middlemiss, 1996) and the potentially deleterious neurodevelopmental effects of excessive intra-synaptic accumulation of serotonin (Lesch and Gutknecht, 2005). Via its intimate relationship with serotonergic signalling, the ADRA2B polymorphism may also exert its epistatic effects via these autoregulatory and neurodevelopmental mechanisms.

Whilst this hypothesis warrants further investigation, the biological epistasis suggested in the present study may have important implications for individual responses to serotonergic and noradrenergic antidepressant drugs. The vast majority of pharmacogenetics studies in depression have focussed on variations in the serotonin transporter (Schosser and Kasper, 2009; Serretti *et al.* 2007). However two large recent projects (STAR*D and GENDEP) have found associations between antidepressant response and a number of candidate genes involved in both serotonin and noradrenaline signalling (Hu *et al.* 2007; McMahon *et al.* 2006; Paddock *et al.* 2007; Uher *et al.* 2009), although none of these studies included the ADRA2B polymorphism. The GENDEP project did examine a polymorphism in the related ADRA2A gene encoding alpha 2A adrenoceptor subtype but failed to find any significant effect despite a previously reported association with the response to the serotonin-noradrenaline reuptake inhibitor milnacipran (Wakeno *et al.* 2008). Of note, neither of the two antidepressant evaluated in GENDEP was a molecular target of the alpha 2 adrenoceptor

group. Thus, the role of polymorphic variation in alpha 2 adrenergic receptors (and their interaction with serotonergic targets) in the therapeutic response of patients with affective spectrum disorders warrants further investigation.

*Study limitations*

The purpose of this study was to investigate epistatic effects of serotonergic and noradrenergic genes on emotional attentional biases. However, the fact that we measured only two polymorphisms out of a number that might contribute to the behavioural effect of interest represents a limitation to this study. Most significantly, we were unable to genotype the additional rs25531 SNP in the long allele of the serotonin transporter gene. However, given that the prevalence of the $L_G$ allele is low (approximately 10%), this is unlikely to have significantly biased our findings. A further limitation is that although the overall sample size was reasonable, there were relatively few individuals in some genotype combinations. This is a particular difficulty inherent to measuring epistatic gene effects (Moore, 2008). Future studies will need to use large sample sizes and evolving methodologies to effectively evaluate the likely effects on emotional processing of multiple gene interactions (Cordell, 2009). The final limitation is that we only used aversive stimuli and male volunteers only. While the latter eliminated possible biases associated with gender differences in emotional processing, it limits the generalisability of our findings. It is therefore unclear whether the observed biases are valence and/or gender specific. Additionally the aversive stimuli used included range of negative emotions (disgust, fear, sadness) rather than specifically dysphoric or threat-related emotions. It is therefore unclear how these processing biases might map onto those considered to relate to depression and anxiety disorders. Further studies with larger sample sizes including men and women will be required to replicate and extend our findings.

*Conclusions*

In spite of these limitations, this study begins to contribute to the understanding of multiple gene effects and interactions in an established cognitive marker for affective spectrum disorders – the negative attentional bias. It further underlines the potential utility of adopting the 'endophenotype' approach in pharmacogenetics studies, i.e., examing the genetic factors underlying not only the clinical response but responses in cognitive and neural markers also. One significant challenge for such studies will be to delineate the interaction between potential neurodevelopmental effects of genetic polymorphisms influencing brain neurotransmitter systems and the acute/sub-acute effects of drug administration. Knockout and transgenic mouse models are likely to be useful in appreciating the dynamics of the behavioural-psychopharmacogenetic-neurodevelopmental interface.

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**Statement of Interest**

The authors declare no conflict of interest.

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**Tables**

Table 1 – Demographic characteristics for 5-HTTLPR and ADRA2B genotypes

| | *5-HTTLPR* | | | | *ADRA2B* | | | |
|---|---|---|---|---|---|---|---|---|
| | S | L | *F* | *p* | Del | Ins | *F* | *p* |
| Number of participants | 34 | 60 | - | - | 51 | 43 | - | - |
| Age [yrs, mean (SD)] | 23.9 (4.9) | 23.9 (4.3) | 0.02 - | 0.88 - | 24.7 (4.9) | 23.0 (4.3) | 1.18 - | 0.31 - |
| IQ [mean (SD)] | 105.9 (6.5) | 104.4 (7.0) | 2.28 - | 0.14 - | 106.7 (5.7) | 104.7 (6.4) | 3.01 - | 0.06 - |

**Figures (titles and legends)**

Figure 1

Title: Schematic Representation of Attentional Blink Task

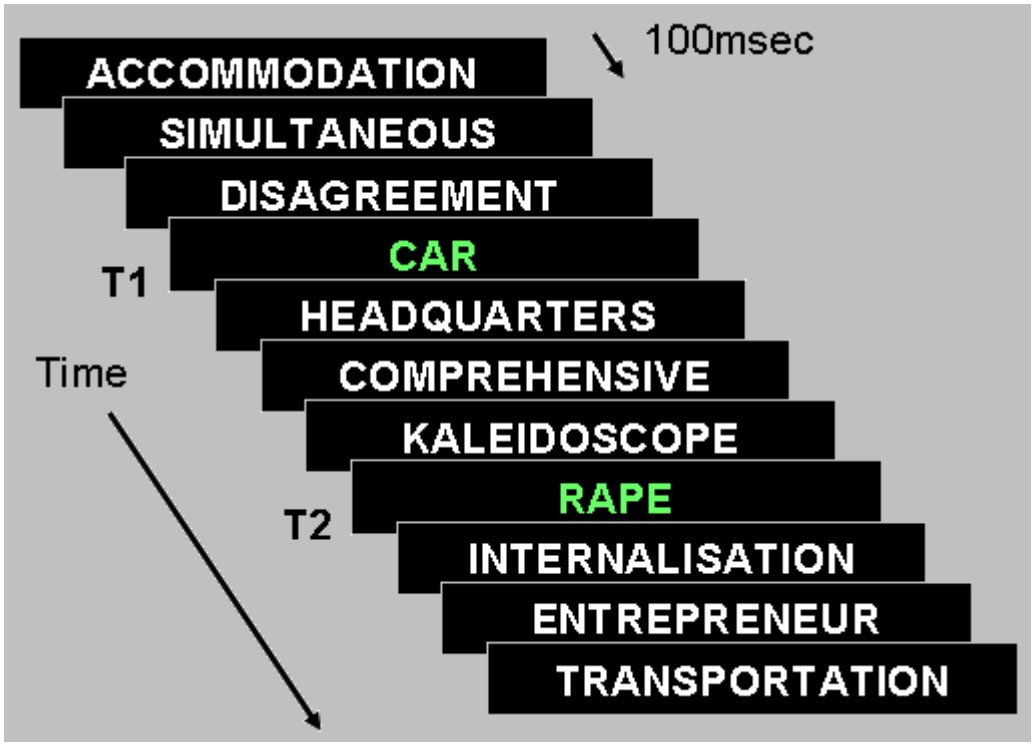
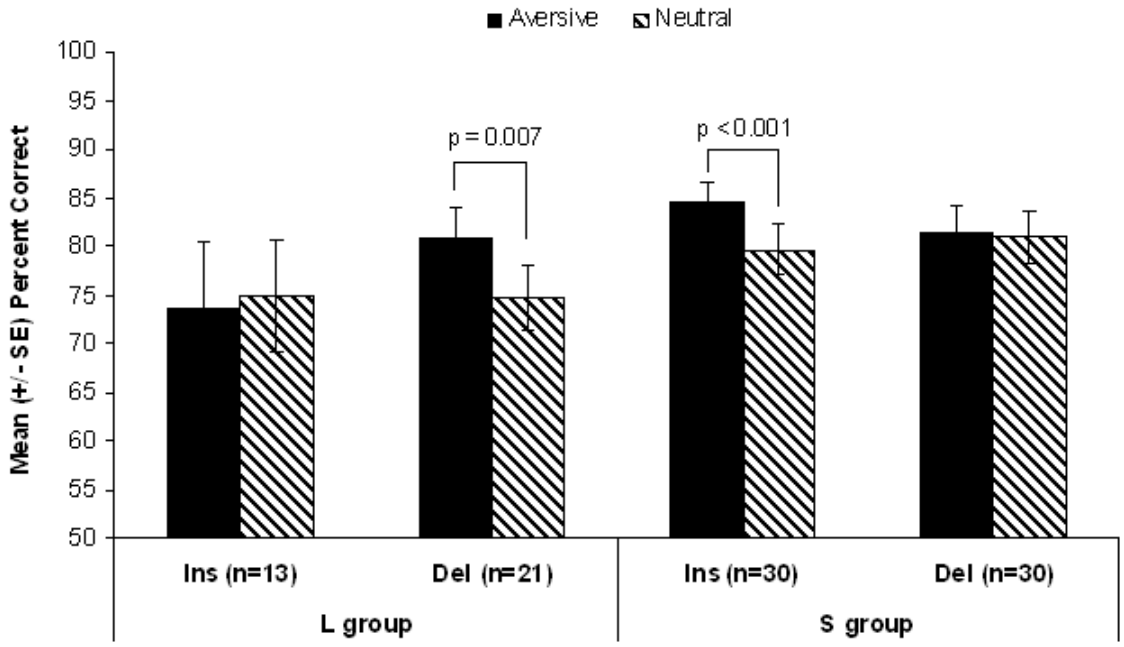


Figure 2

Title: Accuracy (percent correct) of T2 Detection in 5-HTTLPR and ADRA2B Groups



# Discussion of Thesis

## *Summary of key findings*

The aim of this thesis was to examine the effects of three genetic polymorphisms (COMT *val*158*met*, 5-HTTLPR *S*/*L* and ADRA2B insertion/deletion) on emotional processing in healthy volunteers. It was hypothesised that the variants of these genes that have been associated with increased monoamine levels (specifically the COMT *met*158, 5-HTTLPR *S*, ADRA2B *Del* and MAOA *L* alleles) would be associated with increased attentional and memory biases for emotional, relative to neutral information whilst the alternate alleles would be associated with reduced emotional processing biases. This thesis comprises three peer-reviewed publications reporting the following key findings.

### Article 1. Deletion variant of α2b-adrenergic receptor moderates the effect of COMT val158met polymorphism on episodic memory performance (Gibbs *et al.* 2010)

The key finding reported in this article was an interaction between the COMT val158met and ADRA2B deletion polymorphisms in relation to memory performance, independent of valence. Abolition of the relative episodic memory impairment in COMT val/val individuals by possession of the ADRA2B deletion variant was observed. No main effects of either polymorphism were observed. However this is the first demonstration of an effect of ADRA2B genotype on episodic memory performance and its interaction with COMT and suggests that genes that do not exert direct effects on cognition might do so indirectly via gene-gene interactions. The absence of any gene x emotion interaction suggested that the hypotheses relating to the genetic modulation of emotional processing were not confirmed. Prior studies demonstrating behavioural effects of the ADRA2B polymorphism on emotional memory have

used free recall testing rather than recognition as used in this case. It is therefore possible that the failure to demonstrate this effect relates to this difference in approach to the evaluation of memory performance. Under the *dual-process signal detection (DPSD)* model, recognition memory is considered to comprise two distinct processes: recollection and familiarity (Yonelinas, 2002). The latter is considered to be a quantitative, memory-strength based process, associated with a "sense of having previously seen". By contrast, recollection is presumed to be a qualitative threshold process, associated with high levels of contextual detail and high confidence judgements, and is considered most closely related to recall performance (Mandler, 1980). This model was therefore applied to the recognition memory data in order to examine whether any gene x emotion interactions might be elicited in relation to the recollection component. The results of this further analysis are reported in Article 2.

### Article 2. Influence of COMT val158met and ADRA2B deletion polymorphisms on recollection and familiarity components of human emotional memory (Naudts *et al.* 2012a)

The key finding reported in this article is a significant effect of COMT genotype on the emotional memory bias with respect to the recollection, but not familiarity, component. Specifically, recollection was significantly greater for emotionally arousing vs. neutral pictures in carriers of the val158 allele compared to met158 carriers. This is the first reported finding of an effect the COMT val158met polymorphism on memory biases for emotional information. However, in contrast to previous studies, there was still no evidence of an effect of ADRA2B on emotional memory biases in relation to recollection or familiarity processes. This may have been due to inadequate statistical power. However, methodological differences other than recall vs. recognition may have been relevant. For example, in the present study, a 1 week interval between encoding and memory testing was used, as opposed the short 10 minute retention interval in prior

studies. It is possible that the ADRA2B-dependent differences observed by others relate to encoding processes, such as attention and perception, influencing short-term memory, as opposed to the consolidation processes contributing to long-term memory. This would be consistent with the functional neuroimaging findings in which ADRA2B genotype-dependent differences in amygdala activation observed during encoding emotional vs. neutral stimuli did not influence subsequent memory (Rasch *et al.* 2009), although this was attributed by the authors to inadequate statistical power to detect behavioural differences. Given that memory was tested at a single time point in their study and the present study, this issue remains unresolved. Further studies will be necessary to clarify the effects of ADRA2B on the affective modulation of attention/perception during encoding and the effects of post-encoding arousal (Todd *et al.* 2011). However, the findings in relation to the genotype dependent effects of attentional biases for emotional stimuli using the attentional blink task may be helpful in this respect. These results are reported in Article 3 below.

## Article 3. Epistasis between 5-HTTLPR and ADRA2B polymorphisms influences attentional bias for emotional information in healthy volunteers (Naudts *et al.* 2012b)

This article reports evidence of a significant epistatic effect between the 5-HTTLPR and ADRA2B insertion/deletion polymorphisms on attentional biases for emotional information using the attentional blink task. Specifically, the attentional bias for aversive information observed in association with the 5-HTTLPR *S* allele was attenuated by possession of the ADRA2B deletion variant, whereas in the absence of the *S* allele, the bias was enhanced by possession of the ADRA2B deletion variant. This is the first study to examine the contribution of the ADRA2B insertion/deletion polymorphism to individual differences in emotional attentional biases and only the second study to explore the genetic basis of the emotional AB effect - cf.,

Munafò and colleagues previously found an association between 5-HTTLPR genotype, smoking status and detection of smoking-related stimuli in an AB task (Munafò *et al.* 2005). The present data identify a cognitive mechanism linking genotype-dependent serotonergic and noradrenergic signalling that may have implications for the development of cognitive markers for affective spectrum disorders as well as therapeutic drug effects and personalised approaches to treatment.

One limitation of the findings reported in this article is that it was not possible to genotype an additional A/G SNP (rs25531) in the long allele of the serotonin transporter gene that has recently been described. This SNP has been found to further influence transcriptional activity such that the G variant of the *l* allele is considered to result in a reduction in transcriptional efficiency to a level similar to that of the *s* allele (Hu *et al.* 2005; Wendland *et al.* 2006). The frequency of this G allele varies with ethnicity however it is relatively uncommon in white European ethnic groups (Hu *et al.* 2006). It is therefore unlikely to have significantly biased our findings.

## *Implications of findings*

Taken together, these findings contribute to increased understanding of the neurobiology of cognitive and emotional processing, specifically the contribution of genetic variation to individual differences in these processes. In addition to the implications for each finding, discussed in the relevant articles, as a whole, this thesis provides evidence to support the hypothesis that biological epistasis is central to the effect of genetic variation on individual differences in cognitive and emotional processing. It delineates two such examples. This is relevant because failure to take these interactions into account in future studies may obscure

effects and contribute to null findings. Such interactions may also provide pointers towards more individualised, and therefore potentially more effective, pharmacological interventions.

For example, the finding that the cognitive disadvantage associated with COMT val158 allele carriage may be ameliorated by possession of the ADRA2B deletion variant suggests that pharmacological interventions that approximate the neurochemical effects of possession of this variant could be particularly beneficial in the enhancement of cognition in COMT val/val individuals. Although conducted in healthy volunteers, this has relevant clinical implications. Cognitive dysfunction is a highly disabling feature of a number of neuropsychiatric disorders including schizophrenia, bipolar affective disorder, depression and attention deficit hyperactivity disorder (ADHD). Developing disease-modifying and symptomatic therapies for cognitive dysfunction is therefore a major goal of drug development (Searles *et al.* 2008). However drug development, particularly central nervous system (CNS) drug development, requires substantial investment, with development costs estimated at over $1 billion USD, a timeline of over 10-15 years and success rates as low as 5-10% (Kola and Landis, 2004). Population segmentation or personalised medicine, i.e. the use of pharmacogenomics and other biomarkers of mechanism to identify individuals most likely to respond, are increasingly considered crucial to drug development strategies to enhance success rates (Wang, 2010).

Such approaches to enhancing cognition have already been demonstrated with the COMT enzyme inhibitor tolcapone. In the first study to demonstrate these effects in humans, Apud and colleagues found a significant drug x genotype interaction on episodic memory performance, such that tolcapone enhanced performanced in val/val genotypes whereas performance in met/met genotypes worsened. (Apud *et al.* 2007). fMRI revealed a significant tolcapone-induced improvement in the efficiency of information processing in the prefrontal cortex during a

working memory test in val/val individuals. These genotype-dependent effects of tolcapone have since been replicated in relation to working memory (Farrell *et al.* 2012). It has been suggested that these differential effects are linked the inverted U shaped curve relationship between pre-frontal catecholamine levels whereby not only too little, but also too much, may impair PFC function. Under this relationship, tolcapone administration shifts val/val individuals up the curve to a more advantageous position of catecholamine levels and PFC function, whilst pushing met/met individuals down the curve to a more disadvantageous position.

COMT is not considered to play a major role in modulating noradrenergic neurotransmission in the PFC however noradrenaline is also crucial to PFC cognitive function and has therefore also represented a target for clinical enhancement of cognitive function (Arnsten, 2004; Nutt *et al.* 1997). However, four recent studies investigating the effect of two noradrenaline reuptake inhibitors, atomoxetine and reboxetine, on cognitive impairment in patients with schizophrenia failed to demonstrate any consistent beneficial effect (Friedman *et al.* 2008; Kelly *et al.* 2009; Poyurovsky *et al.* 2009; Sacco *et al.* 2009). These inconclusive findings may be due to the fact that all of these studies were small, with participant numbers ranging from 12 to 33, and therefore likely to have been underpowered. However, genotype dependent effects may also have contributed to this inconsistency in a number of ways. This thesis suggests that increased PFC noradrenaline levels as a result of possession the ADRA2B deletion variant may compensate for reduced dopamine levels in COMT val/val individuals. Clinical trials examining the effect of atomoxetine on cognitive dysfunction in healthy individuals and patients with schizophrenia based on COMT genotype are already under way (www.clinicaltrials.gov). However the findings reported in Article 1 of this thesis suggest that such genotype-dependent therapeutic drug effects may be dependent on both COMT and ADRA2B genotypes. For example, noradrenaline reuptake

inhibitors may be most effect in treating cognitive dysfunction in COMT val/val individuals who do not possess the ADRA2B deletion variant.

The findings from Article 3 further support this approach in the treatment of depression (Naudts *et al.* 2012b). These findings suggest that the attentional bias for aversive information observed in association with the 5-HTTLPR *S* allele, known to be associated with genesis and maintenance of depressive symptoms, was attenuated by possession of the ADRA2B deletion variant. Conversely, in the absence of the *S* allele, the bias was enhanced by possession of the ADRA2B deletion variant.

That such biological epistasis exists between serotonergic and noradrenergic genes, is consistent with the fact that these neurotransmitter systems are intimately connected in the central nervous system (de Boer, 1995). There is evidence that presynaptic inhibitory α2 adrenergic receptors are not only involved in the modulation of noradrenergic neurotransmission, but also serotonergic transmission presynaptic heteroreceptors (Clement *et al.* 1992; De Boer *et al.* 1994; Mongeau *et al.* 1993). Yet precisely how these systems may interact to produce the intermediate phenotypes and the clinical disorders themselves remains unclear. The fact that the majority of drugs used to treat affective spectrum disorders act by inhibiting the serotonin transporter seems at odds with the fact that individuals with genetically influenced reductions in serotonin transporter function have greater risks of developing these disorders, as well as poorer treatment response rates (Lesch and Gutknecht, 2005). This ostensible contradiction is increasingly understood in terms of the complex autoregulatory processes governing serotonergic function (Routledge and Middlemiss, 1996) and the potentially deleterious neurodevelopmental effects of excessive intra-synaptic accumulation of serotonin (Lesch and Gutknecht, 2005). Via its intimate relationship with

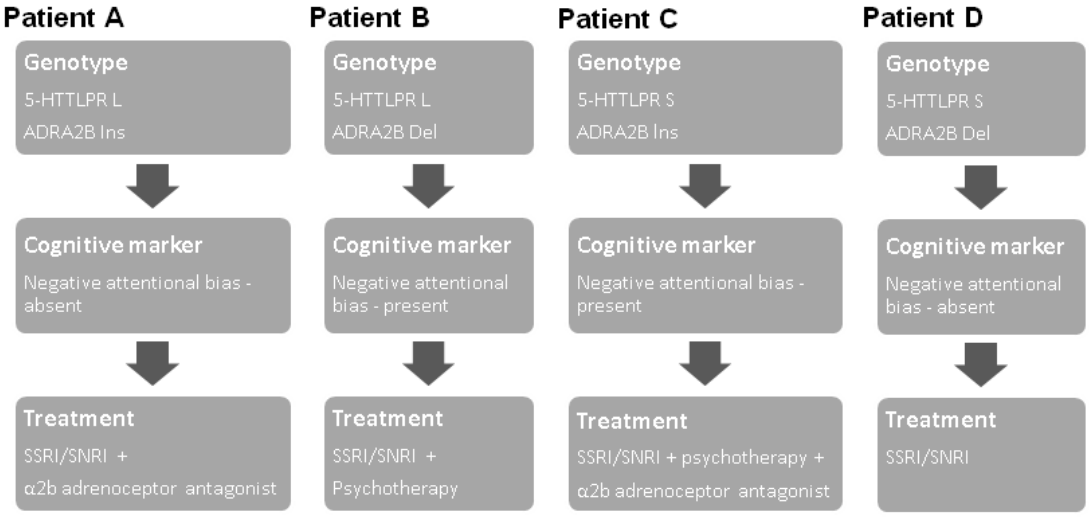
serotonergic signalling, the ADRA2B polymorphism may also exert its epistatic effects via these autoregulatory and neurodevelopmental mechanisms.

Furthermore, it is widely perceived in clinical practice that certain patients are more likely to benefit from noradrenergic, as opposed to serotonergic, antidepressants than others. The mechanisms underlying this effect are not known although it does not appear to be attributable to severity (Wiles *et al.* 2011). However, the established role of noradrenaline in the pathophysiology of depression led to the development of a number of antagonists of the α2 adrenergic receptor aimed at increasing noradrenaline levels by disrupting feedback mechanisms. These compounds all failed in clinical trials for depression in the 1980s. Furthermore, despite its approval for the treatment of depression in Europe, the efficacy of the noradrenaline reuptake inhibitor reboxetine as an antidepressant has been called into question and indeed in the USA the application was rejected after an initial preliminary approval (Dirk *et al.* 2010; Page, 2003). It is possible that the genetic polymorphisms in noradrenergic genes may contribute to these inconsistencies.. The vast majority of pharmacogenetics studies in depression have focussed on variations in the serotonin transporter (Schosser and Kasper, 2009; Serretti *et al.* 2007). However recent studies suggest that variations in the noradrenaline transporter gene may predict response noradrenaline reuptake inhibitors (Hu *et al.* 2007; McMahon *et al.* 2006; Paddock *et al.* 2007; Uher *et al.* 2009). Thus, variation in the α2 adrenergic receptor genes may also be relevant. However, the two studies investigating the ADRA2A gene encoding α2A adrenoceptor subtype have produced conflicting reports (Wakeno *et al.* 2008).

The findings reported in Article 3 also suggest that epistatic effects may be more important in mediating the therapeutic response to both psychological and pharmacological treatments for

depression. Figure 1 below illustrates how these effects might theoretically be used in a personalised approach to the treatment of depression. Such hypothesis-driven population segmentation approaches to are likely to enhance success of clinical trials however their incorporation into pharmacogenomics studies remains in its infancy.

**Figure 1 - Example of how cognitive marker- and genotype-based treatments may be applied for affective spectrum disorders**




**Patients A and C have different 5-HTTLPR genotypes but both have the ADRA2B insertion variant associated with enhanced agonist-mediated down-regulation of monoamine levels. Therefore an alpha 2 adrenergic antagonist may augment, and potentially hasten the therapeutic efficacy of SSRI/SNRI treatment by blocking normal inhibitory feedback mechanisms. Patient A has the 5-HTTLPR L genotype associated with enhanced response to therapeutic drug treatment and no cognitive bias therefore psychotherapy may not offer additional benefits. However Patient C has the 5-HTTLPR S genotype associated with poor treatment response and a cognitive bias that may benefit from adjunctive psychotherapy. Patients B and D both have the ADRA2B deletion variant associated with reduced agonist-mediated down-regulation of monoamine levels and therefore possess a genotype-related constraint on inhibitory feedback mechanisms. Thus, alpha 2 adrenergic antagonist augmentation is not warranted. However Patient B possesses a cognitive bias that may benefit from psychotherapeutic intervention. This algorithm remains speculative but provides an initial example of how the effects of genetic variations and epistatic interactions may guide therapeutic decision-making.**

## *Limitations of thesis and future work*

The studies conducted as part of this thesis have produced preliminary published findings supporting potentially clinically relevant epistatic effects. However, there are a number of potentially relevant unpublished results that need to be considered. Due to the high failure rate for MAOA genotyping, it was not possible to include this polymorphism in the analysis hence this polymorphism is not included in the published data. The inclusion of BDNF *val*66*met* genotype as a covariate did not alter any main effects or interactions hence it was not reported on. All three candidate genes (COMT, ADRA2B and 5-HTTLPR) were included in the planned ANOVAs for both emotional memory and attentional blink tasks however no statistically significant 3-way epistatic interactions emerged. It is possible that the detection of such effects between all three candidate genes in a single analysis would have been hampered by the small sample size and consequent lack of statistical power. However, significant or near-significant 2-way epistatic interactions were observed for episodic memory (COMT x ADRA2B, $p = 0.144$) and attentional bias (5-HTTLPR x ADRA2B, $p < 0.001$). We therefore chose to focus the analysis and three publications exclusively around these specific 2-way gene-gene interactions for memory and attention.

Further work to replicate and extend these findings is required. A larger sample and additional methodology may be required to fully address the issue of epistasis. For example, the traditional parametric statistical linear regression method used in the present studies is not suitable for epistasis due to the potential sparseness of the data, i.e., the high number of multilocus genotype combinations that have few or no data points and as each additional polymorphism is considered, the number of multilocus genotype combinations goes up exponentially such that exponentially larger sample sizes are needed to have enough data to estimate the interaction effects (Moore,

2008). This leads to reduced power and increased type I and II errors. A number of alternative approaches to analysis of genetic epistasis have been developed as a result. One such approach is the restriction partition method (RPM) (Culverhouse *et al.* 2004). An initial step might be to use this approach in the present dataset to improve estimation of the sample size required.

Finally, whilst one advantage of the candidate gene approach used in the present investigation is that it is hypothesis driven, the pilot nature of the study and the limited resources, resulted in the restriction of the investigation to four polymorphisms relevant to the hypotheses of interest. A number of additional potentially relevant genetic variants have not yet been investigated in relation to emotional processing. As there is therefore no existing evidence of a link to emotional processing, they fell outside the scope of this study. For example, genetic variants involved in catecholamine reuptake mechanisms (such as the noradrenaline and dopamine transporters) and dopamine and serotonin receptors were not investigated. Similarly, other known functional polymorphisms affecting adrenergic receptors including the α2 adrenergic receptor subtypes A and C were also not included. These and other genes are likely to be involved in the complex traits under investigation. Emerging hypothesis-free strategies such as Genome Wide Association Studies (GWAS) may be used to address this limitation in future studies however this approach typically necessitates even larger sample sizes and remains impractical for the detection of higher-order (beyond 2-way) interactions such as the 4-way interactions intended to be examined in this study. Therefore the most effective use of GWAS approaches would be in the full characterisation of single gene effects that can be used in large scale, suitably powered, candidate gene studies to determine hypothesis-driven higher order interactions. Nevertheless, in spite of these limitations, this thesis lays the foundations for an approach to investigating genetically determined interactions in neurotransmitter systems influencing emotional processing that may also have implications for vulnerability to affective disorders and antidepressant response.

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# Appendix I

## Medical history checklist

Before you take part in the study we need to know whether you have had any physical or mental health problems. Your responses will be strictly confidential.

**1. Have you ever attended a hospital out-patient appointment?** **YES** **NO**

If yes, specify problem:…….

**2. Have you ever been admitted to hospital?** **YES** **NO**

If yes, specify problem:…….

**3. Do you suffer from any mental health/brain disorder?** **YES** **NO**

O Depression
O Anxiety
O Psychosis
O Mania
O Epilepsy
O Other: …….

**4. Do you regularly use any illicit drugs?** **YES** **NO**

**5. Have you had an alcohol or drug addiction problem in the past?** **YES** **NO**

**6. Do you smoke cigarettes?** **YES** **NO**

If yes, how many: ……per day

**7. Do you take any regular medication?** **YES** **NO**

If yes, specify: ……..

## Summary

It is increasingly recognised that inter-individual variation in responses to emotional stimuli may contribute not only to differences in vulnerability (and resilience) to emotional disorders, but also response to therapeutic agents. Evidence to date suggests that the genes involved in monaminergic neurotransmission (5-HTTLPR, ADRA2B, MAOA and COMT) contribute to differences in emotional processing in healthy individuals. However, little is known about how the different variants of these genes might interact in exerting their effects on emotional processing. The studies reported in this thesis used cognitive tasks tapping into attentional and memory biases for emotional information to examine the contribution of these genes (and their interactions) to emotional processing in healthy individuals. Evidence was found for interactions between COMT and ADRA2B in predicting episodic memory performance and between 5-HTTLPR and ADRA2B in predicting attentional biases for emotional information. These findings warrant further investigation however they contribute to improved understanding of the interplay between monoaminergic genetic variants in emotional processing which may have implications for pharmacological approaches to treating cognitive dysfunction and emotional disorders.

## Samenvatting

Het wordt in toenemende mate erkend dat de inter-individuele variatie in respons tot emotionele stimuli lijkt bij te dragen tot verschillen in kwetsbaarheid (en weerstand) voor emotionele stoornissen en therapeutische respons. Onderzoek wijst er op dat de genen die betrokken zijn in mono-aminerge neurotransmissie (5-HTTLPR, ADRA2B, MAOA and COMT) bijdragen tot verschillen in emotional processing in gezonde personen. Het is echter niet goed gekend hoe de verschillende varianten van deze genen interageren in de uitoefening van hun effect op emotional processing. In dit doctoraat gebruikten we cognitieve taken voor aandachts- en geheugenbiases voor emotionele informatie om de bijdrage van deze genen en hun potentiele interacties in emotional processing verder te onderzoeken. In onze groep gezonde vrijwilligers, vonden we interacties tussen COMT en ADRA-2B met effect op episodisch geheugen en interacties tussen 5-HTTPLR en ADRA-2B met effect op aandachtbiases voor emotionele informatie. Deze bevindingen behoeven verder onderzoek, maar ze dragen toe tot een verbeterd begrijpen van de rol van en interacties tussen de verscheidene genen en hun varianten in emotional processing. Op lange termijn heeft dit mogelijks implicaties voor de pharmacologische behandeling van cognitief dysfunctioneren en emotionele stoornissen.

## Thank you

Thank you Prof van Heeringen. For taking the time to talk psychiatry to me when I was a young, big-eyed medical student taking my first steps around 'K13'. As soon as I had read that first stack of research papers and books on biological psychiatry, I knew there was no turning back: academic psychiatry it was! Thank you so much for the mentorship over the years; for the support when I decided to move across the channel; for your kindness when difficult times hit; and for being my promotor.

Thank you to my mentors in London: to Prof Gunn for the inspiration; to Prof Taylor for her clinical excellence; to Prof Hodgins for her academic brilliance; and to Dr Gibbs for offering all of this in spades, and then some…

Thank you too to some of the fine minds and friends I was lucky enough to come across over the years: Mai Cao-Van, Marianne De Leenheer, Alex Heyse, Fritz Van Durme, Filip Claus, Joris Van den Berghe, Willem Janssens, Liesbet Ovreeide, Ricardo Sainz-Fuertes, Thomas Fahy, Nigel Blackwood, Shubulade Smith and Paul Jessop. I need to make special mention here of my friends Filip Standaert and Bart Stuyts, for their particular kindness and support over the last few years. It mattered.

A huge thank you to my family. To my brothers Patrick and Eric for showing the way; to my mother for her support; to my sisters Mia and Greta for their patience and listening. To my nephews Jonas and Cedric and to my brother-in-law Kris. To my unexpectedly deceased brother, Jan.

Finally, of course, Marthe and Andreas, my beloved children. It's all worth it.

# Curriculum Vitae

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**CURRENT POSITIONS**

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**Institute of Psychiatry (IoP) at the Maudsley, King's College London:** Honorary Lecturer

**Clinical:**

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**PREVIOUS POSITIONS**

**Academic:** my research interests lie in pharmacology, genetics and forensic psychiatry

**Institute of Psychiatry, King's College London:**

October 2003 – April 2009: Deputy Director Forensic Psychiatry Teaching Unit

October 2004 – September 2008: Programme Leader MSc in Forensic Psychiatry

**Ghent University (Belgium):**

October 2004 – September 2010: Academic Consultant

**Clinical:** I worked in a variety of clinical settings across the socio-economic spectrum

**Churchill London Clinic:** low security rehabilitation and psychiatric intensive care

October 2006 - January 2009: Consultant Psychiatrist and Medical Director

**South London and Maudsley NHS Trust:** medium and high security services, prison

November 2003 - September 2006: Honorary Consultant Forensic Psychiatrist

**HIGHER QUALIFICATIONS**

**Core:**

**Consultant Forensic and General Adult Psychiatrist**
Ghent University, Medical School (2003)

**Medical Doctor**
Ghent University, Medical School (1998)

**Additional:**

**Certificate of Teaching Proficiency**
King's Institute of Learning and Technology (KILT), King's College (pending, 2012)

**Intensive Course in Epidemiology and Medical Statistics**
London School of Hygiene and Tropical Medicine (2006)

**Personal Tutor Certificate**
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**Postgraduate Diploma in Forensic Psychiatry**
Institute of Psychiatry, King's College (2002)

**Postgraduate Diploma in Forensic Psychiatry and Psychology**
Catholic University Leuven, Ghent University, Brussels University, Antwerp University (2002)

**Postgraduate Certificate in Family Therapy**
Antwerp University (2001)

**Statistical Methods for Genetic Studies**
North Atlantic Treaty Organisation (NATO) (1998)

**PRIZES**

Belgian College of Neuropsychopharmacology Lundbeck Grant (runner-up) (2003)

International Bursary Royal College of Psychiatrists (2003)

Essay Prize Postgraduate Diploma in Forensic Psychiatry, Institute of Psychiatry, King's College (2002)

Graduation Medal for Outstanding Scientific and Social Merit, Medical School, Ghent University (1998)

**RESEARCH:**

**Papers:**

*Influence of COMT val158met and ARA2B delection polymorphisms on recollection and familiarity components of human emotional memory.*
KH Naudts, RT Azevedo, AS David, C van Heeringen, AA Gibbs
**Journal of Psychopharmacology 2011: 26:819-829**

*Epistasis between 5-HTTLPR and ADRA2B polymorphisms on a cognitive marker of affective spectrum disorders.*
KH Naudts, RT Azevedo, AS David, C van Heeringen, AA. Gibbs.
**International Journal of Neuropsychopharmacology 2012: 15:1027-1036**

*Deletion variant of α2b-adrenergic receptor moderates the effect of COMT val$^{158}$met polymorphism on episodic memory performance*.
AA Gibbs, KH Naudts, RT Azevedo, AS David
**European Neuropsychopharmacology 2010: 20:272-275**

*The effect of amisulpride on emotional memory using a dual process model in healthy volunteers.*
AA Gibbs, KH Naudts, E Spencer, AS David
**Journal of Psychopharmacology 2010: 24: 323-331**

*Quetiapine treatment and improved cognitive functioning in Borderline Personality Disorder.*
F Van den Eynde, S De Saedeleer, KH Naudts, J Day, C Vogels, C van Heeringen, K Audenaert
**Human Psychopharmacology: 2009: 24:646-649**

*Efficacy of quetiapine for impulsivity and affective symptoms in Borderline Personality Disorder.*
F Van den Eynde, V Senturk, KH Naudts, O Thas, C Vogels, K Audenaert, C van Heeringen
**Journal of Clinical Psychopharmacology 2008: 28: 147-155**

*Sexually dimorphic changes in the amygdala in relation to delusional beliefs in first episode psychosis.*
AA Gibbs, P Dazzan, KD Morgan, KH Naudts, C Morgan, G Hutchinson, P Fearon, J Leff, RM Murray, AS David
**Journal of Psychiatric Research 2008: 42: 913-919**

*The role of dopamine in attentional and memory biases for emotional information*
AA Gibbs, KH Naudts, EP Spencer, AS David
**American Journal of Psychiatry 2007: 164: 1603-1609**

*Prison Psychiatry*
N Konrad, J Arboleda-Florez, AD Jager, KH Naudts, J Taborda, N Tataru
**International Journal of Prisoner Health 2007: 3: 111-113**

*Tourette's disorder mimicking an eating disorder.*
F Van den Eynde, V Senturk, KH Naudts,
**Turkish Journal of Psychiatry 2007: 18: 375-378.**

*Neurobiological Correlates of Violent Behaviour among Persons with Schizophrenia.*
KH Naudts, S Hodgins
**Schizophrenia Bulletin 2006: 32: 562-572**

Schizophrenia and violence. A search for neurobiological correlates.
KH Naudts, S Hodgins
**Current Opinion in Psychiatry 2006: 19: 533-538**

*Euthanasia and psychiatry.*
KH Naudts, C Ducatelle, J Kovacs, KR Laurens, F Van den Eynde, C van Heeringen
**British Journal of Psychiatry 2006: 188: 405-409**

*Psychiatry in the Republic of Belarus*
N Golubeva, KH Naudts, S Golubeff, E Evsegneev
**International Psychiatry 2006: 3: 11-13**

*HIV and mental disorders*
K. Jordaens, KH Naudts, F Van den Eynde, M. vervaet, D Vogelaers, K. Audenaert, C Vanheeringen,
**Tijdschrift voor Geneeskunde 2006: 62: 89-99**

*Belgium and its internees. A problem for human rights and a stimulus for service change.*
KH Naudts, P Cosyns, T McInerny, K Audenaert, F Van den Eynde, C van Heeringen
**Criminal Behaviour and Mental Health 2005: 15(3): 148-153**

*Olanzapine in Gilles de la Tourette syndrome: beyond tics.*
Van den Eynde F, Naudts KH, De Saedeleer S, van Heeringen C, Audenaert K.
**Acta Neurologica Belgica 2005: 105(4):206-211**

*Depression in palliative care*
K Audenaert, K Godfrin, F Van den Eynde, KH Naudts, M Vervaet, C van Heeringen
**Tijdschrift voor Geneeskunde 2005: 61(7):530-538**

*Euthanasia and psychiatry: Current situation in Belgium within the international context.*
C.Ducatelle, KH Naudts, K Godfrin, F Van den Eynde, D Van den Abbeele, K Audenaert, M Vervaet, C van Heeringen
**Tijdschrift voor Geneeskunde 2005*:* 61(2):83-96**

*Treating pathological gambling with sertraline.*
F Van den Eynde, KH Naudts
**Tijdschrift voor Psychiatrie 2005, 47: 713-714**

*Abuse and dependence of OTC available codeine and ephedrine containing cough syrups by psychiatric patients*
KH Naudts, MJW Vreeling, F Van den Eynde, E Braxel, K Audenaert, C van Heeringen
**Tijdschrift voor Geneeskunde 2004: 60(18):1303-1309**

*Mental disorders and violence.*
KH Naudts, KEL Jordaens, F Van den Eynde, L Ovreeide, K Audenaert, C van Heeringen
**Tijdschrift voor Geneeskunde 2004: 60(13):934-940 - Belgium**

*The victim/offender dichotomy in psychiatry.*
KH Naudts, K Dhondt, F Van den Eynde, M Vervaet, K Audenaert, C van Heeringen
**Tijdschrift voor Psychiatrie 2004: 4:219-227 – The Netherlands**

**Book chapters:**

*Paedophilia and Brain Scanning: A Fruitful Approach?*
KH Naudts, PJ Taylor, AA Gibbs, F Khalid, F Van den Eynde, C van Heeringen
**Forensic Psychiatric Research Trends (Ed RC Brown) 4: 117-133**
**Hauppage, New York: Nova Publishers 2008**

*Violence and suicide*
KH Naudts, F Van den Eynde
**Handbook Suicidal Behaviour (Ed C van Heeringen) 6: 93-109**
**Utrecht, The Netherlands: De Tijdstroom 2007**

*The Neurobiology of Psychopathy*
C Herba, S Hodgins, N Blackwood, V Kumari, KH Naudts, M Phillips
**The Psychopath. Theory, Research and Practice (Eds H Hervé and JC Yuille) 253-283**
**Mahwah, New Jersey: Lawrence Erlbaum Associates 2006**

*Functional neuroimaging in eating disorders*
F Van den Eynde, S De Saedeleer, KH Naudts, M Vervaet, A Otte, K Peremans, I Goethals, C van Heeringen, R Dierckx, K Audenaert
**Nuclear Medicine in Psychiatry (Eds A Otte et al) 25:407-424**
**Berlin, Germany: Springer Verlag 2004**

*Functional brain imaging in personality research and personality disorder*
K Audenaert, I Goethals, K Peremans, A Otte, F Van den Eynde, KH Naudts, M Vervaet, RA Dierckx, C van Heeringen
**Nuclear Medicine in Psychiatry (Eds A Otte et al) 27:457-474**
**Berlin, Germany: Springer Verlag 2004**

*The use of functional brain imaging in court*
SS Shergill, KH Naudts, J Gunn
**Nuclear Medicine in Psychiatry (Eds A Otte et al) 33: 539-546**
**Berlin, Germany: Springer Verlag 2004**

**Posters:**
I have presented more than 20 posters internationally.

**Book reviews:**
*Surviving Stalking (Pathe, 2002)*
KH Naudts
**The International Journal of Social Psychiatry 2007 Vol 53: 288**

*Evaluating Sex Offenders (Doren, 2002)*
KH Naudts
**Criminal Behaviour and Mental Health 2006 Vol 16(2): 130-131**

*Forensic Management of Sexual Offenders (Prentky, 2000)*
KH Naudts
**Criminal Behaviour and Mental Health 2002 Vol 12 (4): 294**

**TEACHING**

As deputy-director, I managed the day-to-day business at the Home Office Forensic Psychiatry Teaching Unit. Formal business meetings were held every 2 weeks with the administrative staff. Strategic and financial planning took place at the monthly Teaching Unit Executive Meetings and activity reports were produced for the semi-annual meetings with the Home Office. I developed the Programme Design Reports for the 2 new MSc programmes at our department and oversaw the approval and implementation of the programmes (November 2003-October 2004). I was the Programme Leader for the MSc in Forensic Psychiatry and the vice-chair of the Exam Board for this course. I also organized numerous day conferences and workshops in the field of forensic mental health science. I set up collaboration with the Expert Witness Institute with resulted in regular joint conferences on relevant legal topics for forensic psychiatrists and psychologists working as expert witnesses.2005. Other work at the Teaching Unit entailed: selection and recruitment of students; Vice-chair Exam Board of MSc; setting exam questions; examining and marking students on this MSc; supervising students of MSc in Clinical Forensic Psychiatry and Psychology; chairing Research Presentations, Academic Journal Clubs, and Clinical Case Conferences; personal tutelage of MSc students; teaching students on our MSc`s; peer reviewing colleagues' teaching; interviewing job applicants; liaising with senior Home Office staff and producing semi-annual reports. I taught on the MSc courses at the IoP and the London School of Economics. I gave more than 40 lectures both nationally and internationally.

**MANAGEMENT AND ADMINISTRATION**

I gained experience of non-clinical management in a variety of roles since medical school:

2003-09: Deputy Director Forensic Psychiatry Teaching Unit (IoP)

2004-08: Programme Leader MSc in Forensic Mental Health Science (IoP)

2006-09: Medical Director (Churchill London Clinic Independent Hospital)
.
2002-03: Specialist Trainees' Delegate at the Medical Council (Ghent University Hospital)

2001-02: Member of the Board of the European Federation of Psychiatric Trainees

1999-2001: President of Psychiatric Trainees' Association (Ghent University)

1996-98: Students' Representative at the Medical School Faculty's Council (Ghent University Hospital)

1996-98: Students' Representative at the Board of Governors (Ghent University)

1996-98: Students' Representative at the Task Force Development of a new Medical Curriculum (Ghent University)

1994-95: President of the Flemish Medical Student Association

**INTERNATIONAL EXPERIENCE**

I speak Dutch (native language), English, French and basic German. I have studied medicine and psychiatry in Belgium and the UK with two short periods in Israel and South-Africa.

Other international experience includes:

Maudsley Forum (annual IoP course for European psychiatrists): I participated in the planning and implementation.(2004-2006). I am particularly proud of obtaining funding from the Psychiatry Research Trust for several three-month placements at the IoP for colleagues from Central and Eastern Europe (2006)

Instigator research and teaching collaboration State Mental Hospital Minsk (Belarus) (2006)

Collaborator in Rus-Nor forensic psychiatry network (Russia-Norway) (2006)

Humanizing Prison Mental Health and Forensic Psychiatric Systems in Georgia (former USSR). Project Proposal 2005-2007. Geneva Initiative on Psychiatry

Instigator research collaboration on First Episode Psychosis with Lisbon University (Portugal) (2005)

Human Rights Monitoring Visit for MDAC (Mental Disability Advocacy Centre – Budapest (Hungary) and consultancy for MDAC project in Estonia (2004-2005)

European Federation of Psychiatric Trainees: throughout clinical training I was an active board member of this organisation (1998-2003)

**MISCELLANEOUS**

I am passionate about current affairs, literature and theatre.